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THE COLOR REACTIONS OF NAPHTHAQUINONE SODIUM-MONOSULPHONATE AND SOME OF THEIR BIOLOGICAL APPLICATIONS

BY

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THE COLOR REACTIONS OF NAPHTHAQUINONE SODIUM-MONOSULPHONATE AND SOME OF THEIR BIOLOGICAL APPLICATIONS.

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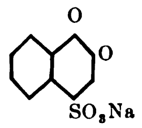
The extraordinary capacity of naphthaquinone-sulphonic acid to enter into reactions with the production of color was first recognized by Witt and Kaufmann,[1] who first prepared the substance by oxidation of amido-naphtha-sulphonic acid. The observations of Witt were recently considerably extended by Ehrlich and Herter,[2] who not only described a number of new color reactions, but also indicated various biological applications which promise to increase our physiological knowledge. Since the publication of these papers I have added a considerable number of new color reactions to those previously observed, and it is my purpose at present to describe some of these. I shall not undertake to discuss fully the chemistry of these reactions, which in many cases is still obscure. I shall, however, describe a number of reactions, selecting especially those which are characterized by sensitiveness, or by some quality which lends the reactions a degree of biological significance.

PROPERTIES OF THE SUBSTANCE.

The 1.2 naphthaquinone 4 sodium sulphonate is an orange-colored powder, which dissolves readily in water. In 95 % alcohol it is slowly and slightly soluble, solution being aided by heat; the solubility in absolute alcohol is still less. In acetone also it is moderately soluble. In ether, chloroform, carbon disulphide, benzene, and petroleum ether the substance is insoluble or very nearly so. The test of solubility in these cases was the failure to obtain any reaction with anilin. The substance is

[1] *Berichte d. Deutschen chem. Gesellschaft*, 1891–2, xxiv, 3157.
[2] *Zeitschr. f. physiolog. Chemie*, 1904, xli, 379.

readily reduced to the corresponding hydro-naphthaquinone compound by means of zinc dust and hydrochloric acid. When thus reduced a reaction with anilin can no longer be obtained. The ease with which reaction occurs in vitro is noteworthy from the fact that the substance undergoes reduction in the animal organism. Efforts to obtain reduction by means of alkaline solutions of glucose were unsuccessful, even when the mixture was subjected to boiling. Under these circumstances the solution becomes a very deep brown, which after boiling a few minutes gives place to a red tint. The reduced and colorless acid solutions of hydro-naphthaquinone sodium-monosulphonate are easily oxidized to the original compound on the addition of potassium persulphate. The proof of this is that the characteristic anilin compound is immediately formed on the addition of anilin to a solution of the hydro compound which has been subjected to oxidation. The chemical constitution of the naphthaquinone sodium-monosulphonate is indicated by the following graphic formula:



The addition of alkali to a watery solution of naphthaquinone compound leads to a gradual darkening of the solution. It is a property of quinones generally that their solutions darken on the addition of basic substances. Possibly this change is connected with tautomerism. This change is greatly accelerated by the use of heat. On rendering the solution acid by means of mineral or organic acids the solution becomes pale yellow. The chemical nature of these changes is not wholly clear. Many substances, when added to an alkaline solution of the naphthaquinone substance, give rise to a dark brown color like that just mentioned. I have found this to be the case in alkaline solutions of uric acid, and with solutions of caffein, xanthin, theobromin, alloxan, etc. I think it probable that the reaction represents merely an acceleration and intensification of the change which takes place when a fairly strong alkali is added to this substance. A similar

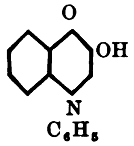
reaction can readily be demonstrated with normal human urine which has been rendered alkaline. If the naphthaquinone compound be added to alkalized urine there is a rapid darkening of the mixture. A dark-colored substance can be readily salted out by means of ammonium sulphate, but its nature is still uncertain. The intensity of the reaction appears to be little diminished by the previous removal of uric acid and other purin substances by means of ammonium salts.

TYPES OF COLOR REACTIONS.

The most striking example of the capacity of our substance to give rise to multitudinous color reactions is seen in the case of compounds which contain an aromatic primary amido group. The substances of this class which react may literally be numbered by the hundreds. For the most part the color reaction obtained in these cases is some shade of red or crimson, but in some instances the color is modified toward brown and usually deepens on the addition of alkali. A second important group, though one which is more limited in the number of its reactions than the preceding, illustrates the so-called acid methylene type. This term refers to such organic substances as possess a methylene (CH_2) group located between two negative radicals, such as CN, COO, C_2H_5, CO NH_2, C_6H_5, CO, $COCH_3$, etc. In all these cases the methylene group becomes labile, and takes on the capacity of making condensation products with our substance. In another group of cases we have reactions with a great variety of aliphatic primary amines, like methylamine and ethylamine. In a certain number of instances, also, we meet with color reactions due to secondary amines, both aromatic and aliphatic. Finally among the organic compounds we meet with some in which the color reaction can scarcely be regarded as depending on a true condensation but is rather to be ascribed to a process of oxidation. This appears to me to be the case with the green color produced by the action of the substance on resorcin.

It seems desirable to describe with some detail certain typical reactions belonging to these various groups of compounds.

Color Reactions of Primary Aromatic Amines.—One of the most striking reactions belonging to this group is that of anilin. This amine undergoes condensation with our substance in neutral solution and without the use of heat. The sensitiveness of the reaction is great, for in a solution of anilin of a concentration of one part in 256,000 parts water there are still indications of the characteristic fire-red precipitate which anilin yields. Even in concentration of one part in 1,000,000 an orange-red color is still perceptible. The constitution of the reaction product has been worked out and is expressed by the following formula.



In this reaction there is an elimination of sodium sulphite and one of the oxygen atoms in the naphthaquinone is replaced by the hydroxyl group.

The reaction with anilin has some biological interest, for it enables us to trace this substance in the organism. The organs of the animal poisoned with anilin are boiled, and a strong watery solution of naphthaquinone is applied to their cut surfaces. The presence of anilin is shown by the development of a red or pink color. Meta- and para-bromanilin give a red precipitate with our substance resembling that obtained with anilin, but there is a falling off in sensitiveness. Metabromanilin ($C_6H_3BrNH_2$) reacts in a solution of 1 part in 256,000; parabromanilin, in a solution of 1 to 16,000. In the case of chloranilin we see a similar difference in sensitiveness in the meta and ortho compounds, the former reacting in solutions of 1 to 250,000 and the latter in 1 to 32,000. The substitution of hydrogen by an alkyl group in the amido group of anilin gives us secondary amines of slight sensitiveness. Methylanilin ($C_6H_5NHCH_3$) and ethylanilin ($C_6H_5NHC_2H_5$) react in 1 part in 8000. The introduction of two alkyl radicals into the amido group of anilin causes complete failure to react. The introduction of a

nitro group into anilin also causes a diminution of sensitiveness, but metanitranilin ($NO_2 C_6H_4 NH_2$) still gives a red color reaction in 1 part in 512,000 and a precipitate in 1 part in 128,000. The ortho and para compounds are much less sensitive. The introduction of two negative radicals gives rise to a great diminution of sensitiveness. This is well seen in dinitranilin (1-2-4), $[(NO_2)_2 C_6H_3 NH_2]$. Three nitro groups, as in trinitranilin $[(NO_2)_3 C_6H_2 NH_2]$, cause a failure to react, at least in neutral solution and in the cold. Methylbenzylanilin ($C_6H_5 N CH_3 C_6H_5 CH_2$) and benzlyanilin ($C_6H_5 NHCH_2 C_6H_5$) are likewise negative. The introduction of a hydroxyl group into anilin in the para position gives us para-amidophenol ($OH C_6H_4 NH_2$), a compound which possesses a considerable degree of sensitiveness. In neutral solution it gives with our substance a fine red, which on the addition of alkali changes to violet or purple. With the aid of alkali it is possible to detect the presence of 1 part of para-amidophenol in 250,000 of water. This reaction acquires a certain medical interest from the fact that para-amidophenol is given off from numerous anilin derivatives which are employed as antipyretics. The presence of para-amidophenol in the urine can be detected by means of the naphthaquinone reaction, but I have not had sufficient experience with the reaction to be able to state whether it possesses any advantage over the tests now in use. The naphthaquinone reaction, however, possesses the interest which arises from our being able to detect readily para-amidophenol in the tissues by means of it.

Of the aromatic diamines two toluylendiamines $[C_6H_3CH_3 (NH_2)_2]$ (1-2-4 and 1-3-4) may be mentioned. Both give red precipitates in neutral solutions. The latter compound (1-3-4) is considerably more sensitive.

As might be expected, toluidine ($CH_3 C_6H_4 NH_2$) and many of its derivatives enter into condensation with the naphthaquinone compound, and the same is true of xylidine $[(CH_3)_2 C_6H_3NH_2]$. Toluidine gives a red precipitate which is detectable in 1 part in 250,000. Methyltoluidine, dimethyltoluidine, and diethyltoluidine fail to react. On the other hand various

nitro-toluidines react, and for the most part readily. The 1-3-6 compound can be detected in 1 part in 236,000; the 1-3-4 compound in a proportion of 1 part in 500,000. The remaining nitro compounds are less sensitive.

Other primary aromatic amines which react well with our substance are benzylamine (C_6H_5 CH NH_2), benzylmethylamine (C_6H_5 CN_2 NH CH_3), a- and b-naphthylamine ($C_{10}H_7$ NH_2), benzidine ($C_6H_4NH_2$ $C_6H_4NH_2$), and phenetidine (H_2N C_6H_4 O C_2H_5). The latter substance can be detected in 1 part in 1,000,000 in watery solution, and in consequence of this sensitiveness its distribution in the organism may be studied with little difficulty. It yields the red color characteristic of aromatic amines and their derivatives.

The sulphonic acid derivative of a-naphthylamine (1-4 naphthylamine-sulphonic acid—$C_{10}H_6$ NH_2 SO_3H) known as naphthionic acid, and much used in the production of dyes, makes salts which react with our substance. Other naphthylamine sulphonic acids (1-5, 1-6, 1-7, 2-5, 2-7) give similar red color reactions, but these substances vary in sensitiveness. The 1-8 compound gives an orange-red. All these substances should be tested in alkaline solution, that is, the solutions of their salts should be rendered alkaline. Naphthylamine disulphonic acid also reacts, but requires both heat and alkali. Amidostilbene disulphonic acid gives a violet color.

When a drop of a 2 % solution of naphthaquinone sodium-monosulphonate is added to a weak solution of phenylhydrazine (C_6H_5 NH NH_2) in water, a purple violet color immediately results without the aid of heat. The addition of potassium hydroxide causes this color to fade. More concentrated solutions of phenylhydrazine yield a red color with our substance, the depth of the red increasing with the concentration of the naphthaquinone solution. One part of phenylhydrazine in 70,000 parts of water still gives the purple color. Benzylphenylhydrazine behaves in a similar manner. Phenylhydrazine oxalate gives a feeble color reaction, but the substance is only slightly soluble in water.

As is well known, anilin forms addition products with a num-

ber of acids. If we take a water solution of one of these addition products, e. g., anilin sulphate $[(C_6H_5NH_2)_2\ H_2SO_4]$, it is found to react with the naphthaquinone compound, even in the presence of an excess of acid. The same is true of the hydrochloric acid compound.

Aliphatic Amines.—Among the aliphatic amines there are some which give distinct reactions with the naphthaquinone compound. In general, substances of this type yield a green color. It is of interest that ammonia itself, when mixed with a solution of naphthaquinone sodium-monosulphonate, gives a green color. Dilute solutions of ammonia give a brown color. As in the case of the aromatic amines, it is the primary amines that give the best reactions; thus ethylamine $(NH_2\ C_2H_5)$ and methylamine $(NH_2\ CH_3)$ react in the cold with a deep green color. Excess of acids causes a change to red. Dimethylamine $[NH\ (CH_3)_2]$, on the other hand, reacts but feebly, with an orange-red color. Triethylamine $[N\ (C_2H_5)_3]$ and trimethylamine $[N(CH_3)_3]$ give no reaction, as might be predicted. Amylamine $[CH_3(CH_2)_3\ CH_2\ NH_2]$ and hexylamine $[CH_3\ (CH_2)_4CH_2NH_2]$ also react with the production of a deep green color. On the addition of acids the former changes to red-orange and the latter to red. Pentamethylendiamine, or cadaverine $(NH_2\ CH_2\ CH_2\ CH_2\ CH_2\ CH_2\ NH_2)$, also gives a green color reaction, which changes to red on the addition of acids. I had no tetramethylendiamine (putrescine) at my disposal, but it appears safe to predict that it will be found to react.

Aromatic and Aliphatic Amidoacids.—It has already been stated that substances containing the amido group are apt to react with the naphthaquinone compound. There is, however, a great difference between the behavior of the amidoacids and the acid amides. The former, as a rule, react readily and give a red color or some shade of brown. The acid amides generally fail to react. Among the aromatic amidoacids may be mentioned in this connection the amidobenzoic acids $(C_6H_4\ NH\ _2CO\ OH)$, methylamidobenzoic acid (para), and amidosalicylic acids. Anthranilic acid (o-amidobenzoic acid) reacts in 1 part in 250,000; and sulphanilic acid $(C_6H_4\ NH_2\ SO_3)$ is not less sensitive. Of

physiological interest is the fact that amidococaine is a reacting substance. Among the aliphatic amidoacids we have glycocoll (amidoacetic acid, $NH_2\ CH_2\ COOH$), alanin (amidopropionic acid, $NH_2\ C_2H_4\ CO\ OH$), and leucin ($C_5H_{10}\ NH_2\ CO\ OH$).[3]

In contrast to the lability of the amidoacids stand the various acid amides: thus, salicylamide ($C_6H_4\ OH\ CO\ NH_2$), benzamide ($C_6H_5\ CO\ NH_2$), phthalamide [$C_6H_4(CO)_2(NH_2)_2$], thiobenzamide ($C_6H_5CS\ NH_2$), toluylamide ($CH_3\ C_6H_4\ CO\ NH_2$), acetamide ($CH_3\ CO\ NH_2$), proprionamide ($C_2H_5\ CO\ NH_2$), lactamide ($CH_3\ CH\ OH\ CO\ NH_2$) fail to react, or react so feebly as to be hardly distinguishable from the controls. Carbopyrrolamide ($C_6H_4N\ CO\ NH_2$) and cyanacetamide ($CN\ CH_2\ CO\ NH_2$) give color reactions in alkaline solutions, the former a green, the latter a purplish red, but these reactions depend on the presence of the pyrrol nucleus and the acid methylene group respectively.

Asparagin ($NH_2\ CO\ CH_2\ CH\ NH_2\ CO\ OH$) reacts with the red-brown color noted in the reactions of the acid amides. Tyrosin ($OH\ C_6H_4\ CH_2\ CH\ NH_2\ CO\ OH$) gives a reddish yellow color deepened by alkali and intensified by acid. Sarcosin ($CH_3\ NH\ CH_2\ CO\ OH$) gives an orange-red in water solution, the color deepening on the addition of alkali.

The color reactions of the amidoacids with the naphthaquinone sodium-monosulphonate suggest the possibility of our being able to follow these substances (at least as a group) in their origin from proteid in the intestine and during their absorption and further distribution.

It should be mentioned here that while carbamide [urea—$CO(NH_2)_2$] and biuret ($NH_2\ CO\ NH\ CO\ NH_2$) do not react, the urea derivatives semicarbazid ($NH_2\ CO\ NH\ NH_2HCl$) hydrochloride and thiosemicarbazid ($NH_2\ CS\ NH\ NH_2$) give red colors with the sulphonate. The latter substance still reacts

[3] Not without interest for physiology is the fact that the hexon bases (diamidoacids) enter into color reactions with the naphthaquinone compound. Preparations of histidine chloride, lysin picrate, arginine nitrate, and ornithin were submitted to me by Dr. Wakeman, who prepared them in Kossel's laboratory. Of these substances, ornithin was found to react most readily. Dilute alkaline solutions of histidine chloride gave an amethyst color. The other hexon bases give the reddish colors usually observed with amidoacids.

when present in the proportion of 1 part in 200,000 if alkali be present. The red which results is a fine crimson, if the naphthaquinone be used in dilute solution. It is destroyed by acid. Diphenylcarbazid also reacts.

Color Reactions with Heterocyclic Compounds.—Among the heterocyclic compounds there are some which give with the naphthaquinone sodium-monosulphonate highly characteristic color reactions of considerable chemical and physiological interest. We have to consider especially pyridine and piperidine (and their derivatives), pyrrol, thiophene, and the pyrazaol derivatives, and finally indol and skatol.

Pyridine (C_5H_5N) either gives no color reaction at all with our substance, or gives a reaction so slight that it is with difficulty distinguishable from a control.[4] On the other hand, the hexahydro compound, piperidine ($C_5H_{10}NH$), gives in water solution a fine scarlet color which gradually fades. The reaction is much hastened by heat. The color is destroyed by alkalies and acids. This reaction is a moderately delicate one, the color being still discernible on the addition of our substance to a solution of 1 part of piperidine in 32,000 parts of water. This reaction with piperidine is probably to be referred to condensation with the labile imide (NH) group contained in this substance, but the chemical nature of the newly formed compound has not been studied.

Of the derivatives of pyridine, the monomethyl compound, picoline, is also negative as regards color reaction. The dimethyl, trimethyl, and tetramethyl pyridines, known respectively as lutidine, collidine, and parvuline, each yield scarlet precipitates with the naphthaquinone compound. These reactions take place most readily in water solutions. Of the derivatives of piperidine, a-pipecolin ($C_5H_9CH_3NH$) gives a fine red, which is destroyed by excess of alkalies; this reaction is facilitated by heat, but takes place in the cold. A-propyl piperidine [coniine ($C_8H_{17}N$)] reacts to our substance to make a deep red color, which is destroyed by acids, but not so readily by alkalies. Another alkaloid, nicotine ($C_{10}H_{14}N_2$), closely related to piperidine,

[4] Kahlbaum's pyridine gave no reaction.

gives a more characteristic reaction with the naphthaquinone sodium-monosulphonate. Even with a small amount of the sulphonate the water solution of nicotine yields a yellow olive-green, which gradually changes to reddish brown. This color is destroyed by acids and alkalies. The reaction is hastened by heat.

Of the members of the pyridine carbonic acid group, the b-compound, nicotinic acid ($N\ C_6H_4CO\ OH$), is the only one which has yet been tried and it proved negative. The members of the furfurane group, including furfurane, furfurole, and pyromucic acid likewise failed to react. On the other hand, pyrrol (C_4H_4NH) was found to give a beautiful and characteristic reaction. If a solution of pyrrol of moderate concentration be treated with one drop of a 2 % solution of the naphthaquinone compound, the solution gradually assumes a pink color which soon changes to purple or violet. The reaction is accelerated by boiling the pyrrol solution before adding the reagent. If the reaction be carried on with the aid of potassium hydroxide, the sequence of colors is somewhat modified, the red tints being prominent at first and gradually changing to purple or violet, if the excess of alkali be considerable. After the color reaction has been obtained with the naphthaquinone, either in watery or distinctly alkaline solution, the addition of acids in excess occasions the development of a yellowish green tint which, after a time, fades. The use of a strong acid, like hydrochloric, occasions the immediate development of the green tint. Regarded as a method of distinguishing pyrrol, this color reaction cannot be said to be remarkably delicate, since in greater dilution than 1 part in 4000 the colors are no longer well marked. Nevertheless, the naphthaquinone color reaction for pyrrol may be regarded as a contribution to our means of detecting this substance. Looked at as a test for the naphthaquinone sodium-monosulphonate, the reaction described is of considerable interest, since even so small an amount as 0.8 of one milligram of our substance yields the typical color reaction with a moderately concentrated pyrrol solution. Of course, we have in anilin a substance with which it is possible to detect small amounts of naphthaquinone sodium-

monosulphonate, but if we take the urine of a rabbit, to which the naphthaquinone compound has been administered, this urine fails to react with a solution of anilin. On the other hand, I have found that such an urine, added to a pyrrol solution which has been boiled, yields the typical colors described, or at least gives a green coloration on the addition of acid. In other words, I have employed pyrrol successfully for the detection of naphthaquinone sodium-monosulphonate in the urine when other reagents have failed. The colored substances formed by reaction of pyrrol with our sulphonate in alkaline solution is readily reduced to a colorless solution by the addition of glucose to the boiling mixture. On the addition of potassium persulphate to the colorless mixture, the leuco body is apparently oxidized to the original colored substance.

The iodine derivative of pyrrol known as iodol [tetraiodopyrrol — (C_4I_4NH)], if dissolved in alcohol and water, reacts slowly with the sulphonate. A blue color results. Alkali should be used in order to get this reaction.

We have in piperidine and its homologues examples of the reaction of the imide (NH) group with the naphthaquinone sulphonate. In pyrrol we have an example of a similar reaction, and this is probably true of pyrrolidine (C_2H_4 C_2H_4 NH) or tetrahydropyrrol (tetramethyleneimide), and of pyrrolidine-a-carbonic acid [5] (C_2H_4 C_2H_3 CO OH NH), a substance which has recently assumed a physiological interest as a cleavage product of proteid material. The discovery of the pyrrol reaction which I have described led me to search for other compounds containing the imide group, and an interesting example of such a substance was found in the cyclic alkylen imide known to the medical profession as piperazine (NH C_2H_4 C_2H_4 NH) and to chemists as diethylenediamine. This substance contains two imide

[5] Dr. Flexner was so kind as to furnish me with the active and inactive copper salt of a-pyrrolidine-carbonic acid prepared by him. A solution of this active salt was not changed by the addition of a drop of the 2 % naphthaquinone solution, but on the addition of potassium hydroxide a well-marked red soon developed, even in the cold. The solutions of the inactive copper salt reacted more slowly under the same conditions and perhaps less fully, an amethyst color being developed as an intermediate stage on the way to brownish red.

groups, and may be looked upon as piperidine, in which the methylene group occupying the para position to the imide group has been replaced by a second imide group. It was interesting to observe that this substance in dilute watery solution reacts very readily in the cold, with the naphthaquinone sodium-monosulphonate with the production of a fine red.[6] No alkali is necessary for the production of this reaction. I have not been able to learn that there is any other color reaction for piperazine. It should be observed further that piperazine reacts much more sensitively than piperidine, which can doubtless be explained through the presence of the second imide group. It will be seen presently that there are two other heterocyclic derivatives, i. e., indol and skatol, which react by virtue of their imide groups. On the other hand, phthalimide [$C_6H_4(CO)_2$ NH] and carbazol (C_6H_4 NH C_6H_4) gave no reactions.

Thiophene.—It may be remarked here that the sulphur homologue of pyrrol, known as thiophene (C_4H_4S), reacts with the naphthaquinone substance in hot alkaline solution, showing a delicate purple which is destroyed by excess of acid.

Pyrazol Derivatives.—In connection with the heterocyclic compounds, I may refer to the reaction noted in some pyrazol ($\overline{\mathrm{HN\ N : CH\ CH\ CH}}$) derivatives. One of the most important of these is phenylpyrazolone ($\overline{\mathrm{HC\ N : NC_6H_5\ CO\ CH_2}}$), which reacts green with our substance and alkali, but soon changes to blue, and on boiling becomes greenish blue. Excess of acids causes a change to yellow-red. Methylphenylpyrazolone reacts similarly. Dimethylphenylpyrazolone ($\overline{\mathrm{CH_3\ C\ N\ CH_3N\ C_6H_5CO\ CH}}$), or antipyrine, does not react, and negative results are also obtained with solutions of dimethylamidodimethylphenylpyrazolone or pyramidon. But these antipyretic drugs can readily be forced to unite with the naphthaquinone sodium-monosulphonate. If we dissolve antipyrine in water it can readily be converted into the green nitroso-antipyrine by the use of sodium nitrite and hydrochloric acid. The nitroso-antipyrine may now readily be reduced to the amido compound by means of zinc and hydro-

[6] Urine to which a small amount of piperazine has been added readily gives this reaction.

chloric acid. But we have seen that amido derivatives of aromatic compounds generally react with our substance. The present instance is no exception, and the typical red reaction is readily obtained. Pyramidon gives similar results.

CONDENSATION PRODUCTS OF THE BENZENE NUCLEUS WITH HETEROCYCLIC NUCLEI.

In this group we have to consider certain quinoline (C_9H_7N) derivatives, and the far more important substances, indol and methylindol, or skatol. Of substances pertaining to the quinoline group, it was found that quinoline and isoquinoline failed to react with the naphthaquinone compound. Oxyquinoline, however, in alkaline solution gave a deep olive-green or green-brown fluid which, on dilution with water, brightened to green. The color is destroyed by acids. Paratolu-quinoline [$C_9H_6(CH_3)N$] in alcoholic solution was found to give a deep red-brown coloration on the addition of a solution of naphthaquinone sodium-monosulphonate. This color was not damaged by moderate excess of acids or moderate excess of alkali.

Of the various color reactions which have been brought to light through experiments with the naphthaquinone derivative, none are of greater physiological importance or of more interest to the chemist than those which relate to the behavior of indol and skatol. Although the color reaction obtained by combining a solution of indol with the naphthaquinone derivative was known to Professor Ehrlich, he never obtained an opportunity to study it, and no mention was made of it in the publication to which I have referred.

The course of the color reaction between indol or skatol and the naphthaquinone compound varies somewhat with the conditions under which the test is carried out. For this reason it is necessary to observe rather closely certain details in order to obtain comparable results. If we add to the fairly dilute solution of indol in water (say 1 part indol in 50,000 of water) 1 drop of the 2 % solution of the naphthaquinone sodium-monosulphonate, no reaction occurs. On the addition of a drop of 10 % solution of potassium hydroxide there gradually develops

a blue or blue-green color, which fades to green on the addition of an excess of alkali. On rendering acid the green or green-blue solution, the fluid assumes a pink color. The development of the color reaction is markedly hastened by heat. If, instead of adding the alkali to the indol solution in the test-tube after the addition of the naphthaquinone solution, one adds the alkali previously to the introduction of our substance, the course of the reaction is somewhat different, provided the concentration of the indol solution be somewhat greater than that already mentioned, and provided also that the reaction be carried on with the aid of heat. Under these circumstances the blue color develops and deepens, but in a short time it becomes evident that the precipitation of the new color compound is taking place. At first the indol compound is separated in fine particles which coalesce to form larger ones, and which possess a spongy appearance, and after a time rise to the surface, leaving the faintly tinted mother liquor. If particles of this blue sponge-like substance be examined with the aid of a microscope, it is found to consist entirely of well-defined acicular crystals, resembling pine-needles in shape and closely felted together. These crystals are blue, and have a diameter of about one micron and a length of from fifteen to forty microns. They are very slightly soluble in water, and considerably more soluble in alkali. The chemical nature of this felt-like substance is not at present wholly clear, but some facts regarding it have been acquired. A considerable quantity of the new compound was made by the method just outlined, collected on a filter, and washed with water. The material thus obtained was dried to constant weight and then subjected to a nitrogen determination by Dr. Wakeman. The proportion of nitrogen contained in the molecule of the new substance was such as would correspond closely to a compound formed by the union of one molecule of naphthaquinone sodium-monosulphonate with two molecules of indol.[7] This result points

[7] The percentage of nitrogen in a compound consisting of two molecules of indol and one of naphthaquinone monosulphonate is 5.669 %. No allowance is here made for the elimination of one molecule of water, which must occur if the above assumption as to the constitution of the new compound be correct. Making this correction the percentage of nitrogen is 5.88 %. The percentage

to the condensation of one of the carbonyl groups in the naphthaquinone compound with the imide group of two indol molecules, as in the condensation of quinone and hydroxylamine to form quinoneoximes. It is difficult to see how the condensation can take place with the elimination of the sulphonic acid group, as occurs in the case of the formation of the naphthaquinone anilid already mentioned.

A highly interesting feature of the condensation product of indol and the naphthaquinone compound is its solubility in chloroform, acetone, and other solvents, with the production of an intense red color. If we cause the formation of the blue color by bringing together indol and our substance in the manner described above, the blue color can be quickly removed by shaking the fluid with chloroform. As the chloroform grows pinkish-red, the blue color disappears from the aqueous solution. This property is of considerable importance in testing for indol, as it serves to distinguish the indol reaction from other reactions which yield a similar color. The relation between the red color of the solvent and the blue color of the compound can be very strikingly exhibited by means of the following experiment. A small quantity of the washed reaction product of indol and the naphthaquinone derivative is dissolved, with or without the aid of heat, in acetone. The color of the acetone is at first red and deepens to purple-red. On diluting with water and adding potassium hydroxide the solution grows blue, and this blue color can readily be transformed to red by shaking out with chloroform, as already described.

The behavior of skatol is very similar to that just described for indol. Strong solutions of skatol yield a blue color on the addition of the naphthaquinone, provided they have been already rendered alkaline. Weak solutions of skatol do not yield a blue color, but give rise to a distinct violet or purple hue. This is the most important feature in the distinction of skatol from indol.

actually found in the new compound was 5.819 %. A further confirmation of the correctness of the above supposition as to the nature of the compound exists in the fact that it contains sulphur, which it could not contain were the condensation to occur as in the case of the union with aniline.

By means of the procedure which has already been described for indol, it is possible to separate the reaction product of skatol and naphthaquinone sodium-monosulphonate. The crystals in this case are of the same form as those described in connection with the indol compound, and are similarly arranged. They are, however, smaller and are violet in color. They are soluble in acetone and chloroform, with a resulting brilliant red color, like that described for the solution of the indol compound. The violet color obtained from skatol and the naphthaquinone derivative can therefore be washed out of ordinary alkaline aqueous solutions by means of chloroform.

The reactions of indol and skatol with the naphthaquinone compound are delicate, and it is possible to detect these substances in alkaline aqueous solutions of about one part in one million parts of water, if suitable precautions are taken in making the test.

Reactions of Phenols.—A considerable number of phenols react with our substance, but only a few will be mentioned here. Common phenol (C_6H_5OH) reacts in alkaline solution with a blue-green color. Orthocresol ($CH_3\,C_6H_4OH$) likewise reacts green, but para-cresol is insensitive, and dinitrophenol [(NO_2)-C_6H_3OH] also fails to react, the acid groups here interfering. Trinitrophenol [$(NO_2)_3C_6H_2OH$], (picric acid), is also negative. Thymol ($C_3H_7\,C_6H_3CH_3OH$) gives a blue-green. Of the dihydroxyphenols resorcin [$C_6H_4(OH)_2$ 1:3] gives a green in alkaline solution, which is fairly sensitive (in 1 part in 30,000 of water). Under certain conditions it is possible to obtain a violet color after the appearance of the olive-green. Hydroquinone [$C_6H_4(OH)_2$ 1:4] reacts brown in the presence of alkalies. Pyrocatechin [$C_6H_4(OH)_2$ 1:2] in alkaline solution gives with the sulphonate a red or olive color (according to the conditions of the reaction), which probably depends on oxidation. A-naphthol gives a green color with our sulphonate, but the reaction is not delicate; b-naphthol does not react. Of the trihydroxyphenols, phloroglucin [$C_6H_3(OH)_3$ 1:3:5] gives a blue in sodium carbonate solution, which alters in a few minutes to blue-violet and on heating gives a dark blue. On the addition of acids the

color changes to yellow. It is a general rule that acids bring about the decoloration of the phenolic color compounds of our substance, a yellow or yellow-red fluid usually remaining.

Pyrogallol (1:2:3) in alkaline solution changes to red on the addition of the sulphonate, but I attribute this to a further oxidation and not to a condensation, for the same result is obtained with oxidizing agents. I have already mentioned that the reaction with resorcin probably depends on oxidation. Oxyhydroquinone [$C_6H_3(OH)_3$ 1:3:4] yields a red-brown in alkaline solution, and is the most sensitive of the three trihydroxyphenols.

Reactions Based on the Acid Methylene Group.—As already stated there are a number of naphthaquinone reactions which depend on condensation with the methylene (CH_2) group. It is, however, chiefly in the case of methylene groups which lie between two negative radicals that these reactions take place. For our present purpose it is not necessary to describe these reactions in detail. The following substances may, however, be mentioned as examples of bodies which enter into reactions of this type: acetylacetone (CH_3 CO CH_2 CO CH_3), benzoylacetone (C_6H_5 CO CH_2 CO CH_3), acetonedicarbonic-ethylester ($CO_2C_2H_5$ CH_2)$_2$ CO CH_2, desoxibenzoin (C_6H_5 CO $CH_2C_6H_5$), cyanacetamid ($CO_2NH_2CH_2$ CN), acetacetic-ethylester (CH_3 CO CH_2 C_2H_5 CO_2), and benzoylaceticester (C_6H_5 CO CH_2 C_2H_5 CO_2). Of these substances acetylacetone and benzoylacetone give red-brown colors. Acetaceticester and acetonedicarbonic ethylester yield orange-red tints. Cyanacetamid gives an immediate red, which deepens in one or two minutes to purple-red, but on boiling develops into a deep red-violet. All reactions referred to in this section are developed in alkaline solution.

Compounds of Hydrocyanic Acid.—The majority of the organic compounds of hydrocyanic acid which have been examined failed to react with naphthaquinone sodium-monosulphonate; thus, proprionitrile (C_2H_5CN), butyronitrile (C_3H_7 CN), mandelicacidnitrile ($C_6H_5CHOHCN$), benzaldehydecyanhydrine ($C_6H_5CHOHCN$), acetonecyanhydrine (C_2H_6COHCN), aldehydecyanhydrine ($CH_3CHOHCN$), and metatolunitrile (CH_3C_6-H_4CN) failed to react. Benzylcyanide ($C_6H_5CH_2CN$) gave a

red-brown reaction with alkali, but the reaction was feeble. Acetonitrile (CH_3CN) gives a similar reaction. Malonitrile [$CH_2(CN)_2$] gives a green color in the cold without alkali. The addition of caustic potash to the green solution gives a deep red which fades slowly. A-naphthonitrile ($C_{10}H_7CN$) reacts with a deep red color in the presence of potassium hydroxide. Orthotolunitrile and paratolunitrile ($CH_3C_6H_4CN$) give red reactions with alkali. It may be mentioned in this connection that guanidine ($CNH<^{NH_2}_{NH_2}$) in alkaline solution gives a violet color with the naphthaquinone compound, while amidoguanidine reacts red. Rhodaninic acid in alkaline solution gives a deep violet.

Reactions with Compounds of the Purin Base Type.—Mention has been made already of the fact that uric acid, xanthin, caffeine, alloxan, etc., in the presence of an excess of alkali cause a rapid browning of the naphthaquinone compound. The nature of the change which the reagent undergoes is not known, but it appears to be of the same nature in all these cases. It appears, moreover, to consist in an accentuation and acceleration of the change which occurs when an alkaline solution of the monosulphonate is permitted to stand.

A reaction of a different sort is observed in the case of a substance related to the purin bases, namely, murexid, the ammonium salt of purpuric acid [$C_8H_4(NH_4)N_5O_6$]. This body contains five imide groups, four of which pertain to the two alloxan radicals which are united through the fifth imide group. It seems likely that it is the presence of this fifth imide group which causes the murexid reaction to differ from the reactions above mentioned.

If one adds to a water solution of murexid in a test-tube one drop of a 2 % solution of our monosulphonate, only a slight yellow coloration occurs in the fluid. But on the addition of three or four drops of a 10 % solution of potassium hydroxide, there gradually develops (without the aid of heat) a fine violet color, which ultimately deepens considerably and then fades. The addition of an excess of naphthaquinone leads to the pro-

duction of a red fluid which may deepen to brown, and which on dilution may assume a green tinge.

I have not been able to find any description of another color reaction for murexid.

Reactions of Substances containing Sulphur.—It was found in the course of experiment that many substances containing sulphur react with the naphthaquinone compound in a characteristic way. The addition of the sulphonate to a solution of sodium sulphide causes the immediate formation of a dark brown (sometimes black) reaction product, which after a few seconds disappears, leaving the solution light red. This behavior is probably due to reduction. By the use of a great excess of the naphthaquinone compound it is possible to obtain a permanent dark brown color. Similar results are obtainable by passing a stream of hydrogen sulphide through a naphthaquinone solution and then adding alkali.

Observations were made on several mercaptans, including benzylmercaptan ($C_6H_5\,CH_2\,SH$), butylmercaptan (C_4H_9SH), and ethylmercaptan (C_2H_5SH). All gave a brown color similar to that which was obtained in the case of inorganic sulphides. It was noticed that when acid was added in excess to a solution in which the brown reaction product had been formed, the brown color gave way to a yellow-green fluid exhibiting opalescence. A similar but less pronounced decolorization opalescence was noticed in the case of ethylmercaptan. Slight opalescence was still noticeable in a water solution of one part of the mercaptan in about 20,000 of water. I think this behavior of ethylmercaptan may prove useful to chemists as an adjuvant to the usual test with a mercuric compound. The opalescence is apparently due to the separation of sulphur. This separation is more marked in solutions of sodium sulphide and ammonium sulphide.

Various sulphur derivatives of urea give a brown color with our sulphonate, the color disappearing rapidly unless a considerable excess of the naphthaquinone compound has been used. But these urea derivatives react only after the employment of heat and alkali.

Various proteids and allied bodies (fibrin, caseine, gelatin) behave like the thioureas, and it may be surmised that their capacity to give this reaction depends on the presence of sulphur in some cleavage product.

The thioureas tested were thiourea [$(NH_2)_2CS$], phenylthiourea ($CS\ NH_2\ NH\ C_6H_5$), and allylthiourea (thiosinamine—$CS\ NH_2\ NH\ C_3H_5$).

In testing for proteids and for thioureas it is of the first importance to make a careful control observation with the naphthaquinone compound, for this gives a brown-red color when boiled with alkali. If we take two test-tubes, one containing a hot-water solution of a definite (considerable) quantity of potassium hydroxide alone, and a second containing proteid which has been boiled with the same amount of potassium hydroxide, and add to each tube one or two drops of the naphthaquinone solution, the reaction of the proteid is obvious and is especially marked in the first seconds. But if the comparison be carelessly made, with excess of the reagent, the reaction may be masked. Red succeeds the original evanescent brown. The proteids give the sulphur (?) reaction even after the boiled solution has been cooled.

Reactions of Proteids.—No thorough study of the reactions of the sulphonate with proteids has been made, but it is certain that such reactions occur. Edestin furnished me by Mr. F. Underhill caused a reddening of the naphthaquinone solution when treated with potassium hydroxide. Merck's mucin yielded a dark brown, and casein (impure) behaved similarly, the color being transient. Witte's peptone (chiefly albumoses) caused some browning of the reagent. Small quantities of proteid have little effect. Crystalline leucylglycyl prepared by Dr. Flexner in the laboratory of Emil Fisher gave a green color in the presence of the monosulphonate, but heat and alkali were required to develop this. In all experiments with proteids it is of the utmost importance that the controls be carefully made, for heat and alkali act on the mono-sulphonate to cause changes in the color of this substance, due to unknown changes in constitution. A study of the proteid group reactions is now in progress.

Unclassified Reactions.—The list of subjects already mentioned as reacting with our sulphonate to yield color reactions may perhaps be fairly regarded as representative, but it certainly is not exhaustive. Reactions have been observed with a number of substances which belong in the categories here adopted, and it is safe to predict that many more will be found in time. Some substances give reactions which it is not possible to classify at present. Thus nitrourethane (NO_2 NH $CO_2C_2H_5$) gives a blue-violet in alkali, trinitrotoluol [CH_3 C_6H_2 $(NO_2)_3$] a brown-red, pyrotartaric acid ethylester (CH_3 CO CO_2 C H_5) a deep green, acetone (CH_3 CO CH_3) a pink color, and Michler's ketone or tetramethyldiamidodiphenylketone [$(CH_3)_2$ N C_6H_4 CO C_6H_4 N $(CH_3)_2$] a deep red in alkaline hot alcoholic solution. This reaction is of no diagnostic value and is difficult to demonstrate.

ON THE METHOD OF USING THE NAPHTHAQUINONE COMPOUNDS.

As much depends upon the way in which our compound is used in making tests, it is desirable in examining any substance with a view to finding whether it gives a color reaction to follow a definite order of procedure and to observe certain precautions. If the substance to be examined is an acid, its solution should be neutralized before adding the naphthaquinone, as a free acid is apt to decolorize any colored body that may be formed. On the other hand, if the solution to be tested is naturally alkaline to litmus, or has been rendered alkaline by the addition of an alkaline carbonate or hydroxide, the important influence of alkali in deepening the color of the naphthaquinone solution must be kept in mind. It is necessary in such instances to make a control observation on a solution of the reagent, to which has been added an amount of alkali comparable to that used in the test. Similarly it is essential to remember that even the weak solutions of naphthaquinone sodium-monosulphonate are greatly deepened in color when boiled in the presence of alkali. A moderately concentrated watery solution of naphthaquinone sodium-monosulphonate assumes a deep red-brown color on boiling with potassium hydroxide. It is therefore important to take account both of the quantity of alkali used, and of the concentration of

the reagent. The nature of the alkali is not a matter of indifference, for many substances, which give a reaction with the naphthaquinone compound in the presence of caustic potash, do not give this reaction in the presence of sodium carbonate.

When the substance to be tested has been brought into solution in water a few drops of a 2 % aqueous solution of the naphthaquinone compound are added. If no color appears a few drops of a 20 % solution of caustic potash are introduced. If there is still no color reaction the mixture in the test-tube may be boiled, and the color which develops is compared with that of the control, made as above mentioned. The effect of acetic acid and of mineral acids should be tried separately upon the alkaline solution as well as upon the neutral mixture. It is not always a matter of indifference whether we add the alkali before or after the introduction of the naphthaquinone compound. Thus in preparing the reaction product of our substance with indol, it was found best to render the indol solution alkaline before introducing the naphthaquinone compound. The concentration of the substance to be tested sometimes exerts a distinct influence on the result of the test. For example, a strong solution of resorcin to which a few drops of a 20 % solution of caustic potash has been added assumes a red color, and this color remains on dilution with water. The addition of potassium hydroxide, however, brings out the characteristic green color. The nature of the solvent must be taken into consideration at times, and the same solvent must be used in making the control observations. In the case of solutions where acetone or a mixture of acetone and water is used, it must be remembered that the naphthaquinone reagent gives a ruby-red or pink color with this ketone. Further details need not be mentioned, as they will suggest themselves.

BIOLOGICAL APPLICATIONS.

In consequence of the properties which have already been described or mentioned, the naphthaquinone sodium mono-sulphonate possesses a number of biological applications. The number of applications known to us at present is few com-

pared to those which experiment will show us to exist. What we know at the present time of the biological uses of the substance can be best discussed under three headings; first, the distribution of the aromatic compounds in the living organism; second, the occurrence of syntheses in the living organism; and, finally, the action of certain naphthaquinone color compounds in the body.

DISTRIBUTION OF AROMATIC COMPOUNDS.

The study of the distribution of those aromatic compounds which react readily with naphthaquinone sodium-monosulphonate is only in its first stage of development; but the experiments already made show that by means of our substance there is much to be learned regarding the relation of the distribution of substances and their chemical constitution. The few experiments which have been made up to the present time relate especially to the distribution of antipyretic drugs. In this class we have the derivatives of anilin, like phenetidine, phenacetine, acetanilid, and paraamidophenol, and, furthermore, pyrazolone derivatives, including antipyrine and dimethylamidoantipyrine or pyramidon. In experiments made recently with Mr. Frederic Bartlett it was found that these various substances could be detected without much difficulty in the liver and kidneys of rabbits a few hours after the administration of fairly large doses. In some instances the quantity found in given weights of liver pulp approximated the quantities obtained from the treatment of the nervous system, if one may judge from the intensity of the color reactions. The preparation of the liver pulp differed somewhat according to the substance sought. Alcohol was used in the extraction of substances which, like phenacetin and acetanilid, are not readily soluble in water. The proteids were precipitated in most instances by means of acetic acid, the filtrate being concentrated and neutralized before the application of the naphthaquinone test. In the case of phenetidine and paraamidophenol which react directly, no further steps were necessary before applying the reagent. Phenacetin and acetanilid, however, do not react directly, and it was necessary to decompose

the molecule by boiling with dilute sulphuric acid before applying the naphthaquinone test. The anilin sulphate thus formed reacts with the sulphonate.

The detection of antipyretics in the cells of the liver, freed as far as possible from blood, is an observation of some clinical interest, because it shows that other parts of the body besides the nervous system take up the substances. This fact should awaken us to the possibility of damaging other parts of the organism than the nervous system, by the indiscreet, long-continued use of analgesic antipyretic drugs.

Among the substances which it would be of interest to trace in the organism by means of our compound may be mentioned the derivatives of salicylic acid and cocaine; whereas salycilamid does not react with the naphthaquinone compound, amidosalicylic acid enters into a color reaction and can probably be traced by means of this. Cocaine does not react with our substance, but on the introduction of the amido group gives us a substance which reacts similarly to aromatic amines. It is true that the anæsthetic action of cocaine is somewhat diminished through this substitution, but since this physiological effect is not destroyed, a knowledge of the distribution of amidococaine in the organism might prove of some interest. It seems reasonable to believe that by the aid of our sulphonate it would be possible to trace the passage of many organic substances into the interior of the eye.

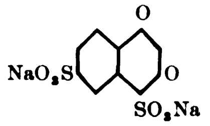
A promising field of investigation appears to me to be the excretion of aromatic compounds through the bile. Of the many substances which react with our sulphonate there are certainly a number, and probably many, which find their way into the bile. The selection from these of the most highly bactericidal and least toxic for the mammalian organism might prove of use in preventing and combating infections of the bile passages.

EXPERIMENTAL SYNTHESES IN THE LIVING ORGANISM.

It is natural that so reactive a substance as the naphthaquinone sodium-monosulphonate should have led to endeavors to bring

about syntheses with the living body. Experiments having this end in view were begun in the laboratory of Professor Ehrlich, and some of these were referred to in the conjoint publication already mentioned. It will not be out of place to refer here to these experiments, and to certain additional ones that have since been undertaken, although the results that have been obtained up to the present represent only a partial degree of success.

Observations made by means of intravenous infusion of the naphthaquinone sodium-monosulphonate gave results that were so little encouraging, that trials were made with the corresponding disulphonate. This substance possesses a second sulphonic acid group in the 6 position, as the following formula indicates:



This secondary sulphonic acid group is not eliminated in the course of ordinary condensations with other substances. The disulphonate is less toxic than the monosulphonate, and confers increased solubility not only on the substance itself, but on its reaction products. The dyes formed through the reactions of the disulphonate assume the character of acid dyes.

The first experiments undertaken were made with anilin with the intention of developing a neutralizing antitoxic action. This undertaking was, however, wholly unsuccessful, for although the red product of condensation could be detected in the bile, there was no evidence of an actual synthesis in the living cells. This fact is in itself of considerable physiological interest, for it indicates that certain cells, like those of the liver, are capable of holding apart substances in spite of the fact that they possess a strong chemical affinity for one another. The explanation of this probably lies in the different destinations of the two substances in the cell territory. This idea seems not improbable when one reflects that the different intracellular enzymes must be conceived to operate in physiologically separate portions of individual cells. The proof that both substances exist side by side

lies, of course, in the presence of their reaction product in the bile. Although a portion of the naphthaquinone compound is undoubtedly reduced in the organism to the corresponding hydronaphthaquinone derivative, my experiments show that a portion of the unreduced substance finds its way as such into the urine. Hence we cannot attribute the failure to obtain a synthesis with anilin to the occurrence of complete reduction in the body.

Better success attended efforts to induce synthesis with the amidobenzoic acids and with a-naphthylamine sodium sulphonate, which, injected into the subcutaneous connective-tissues or into the muscles simultaneously with the infusion of a 2 % solution of naphthaquinone sodium-disulphonate, is seen to be followed by a reddening of the structures about the seat of injection. The best results were obtained with o-amidobenzoic (anthranilic) acid. This reddening depends on the formation of the reaction product of the sodium anthranilate (or other aromatic amido compound) with the naphthaquinone derivative. In another set of experiments partial syntheses were induced in the living cells, if we may judge from the coloration of the tissues. The most successful results were obtained by infusing a solution of the naphthaquinone sodium disulphonate in one vein, while a solution of a neutralized amidoacid was infused in the corresponding vein on the other side; the two solutions being alternately infused. But although some degree of success was attained in this way, it could easily be shown that small portions of the substances entered into reaction with each other. This was indicated by the fact that, if the mixture of the substances under consideration was affected outside the body previous to infusion, the coloration of the cells was considerably deeper than in the cases where these substances were invited to unite within the living organism.

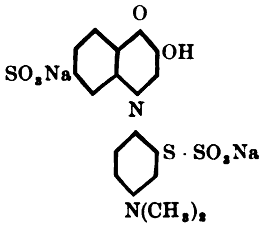
A number of experiments have been made with a view to determining whether a synthesis between indol and the disulphonate is effected within the living organism. Experiments of this sort are of considerable physiological importance on account of the part played by indol in connection with certain human cases of excessive intestinal putrefaction. The evidence indicates

that a partial synthesis does actually occur in the organism. The most significant facts bearing on this point are the following. If a rabbit be infused intravenously with a saturated watery solution of indol, in such a way that from $\frac{1}{20}$ gm. to $\frac{1}{10}$ gm. of indol be introduced in the course of about thirty minutes, the animal develops fibrillar twitching (chiefly about the face); shows a greatly increased excitability of the reflexes and secretes urine containing an abundance of indoxyl salts. If, however, such an infusion of indol be accompanied by a simultaneous intravenous infusion of $\frac{1}{20}$ gm. to $\frac{1}{10}$ gm. of naphthaquinone sodium disulphonate in watery solution, the fibrillation caused by indol alone does not appear, nor is the excitability of the reflexes heightened. Furthermore, there is only a moderate increase in the indoxyl compounds of the urine. There are, in such cases of simultaneous infusion of the disulphonate and indol, a temporary suppression of urine and some diarrhœa. The suppression may be followed by the appearance of hæmoglobin and granular casts in the urine. Apparently similar conditions may be induced by the intravenous infusion of a solution of the crystalline reaction product which has been already described in connection with the discussion of indol.

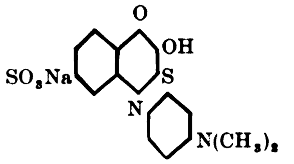
Whether the detoxicating influence of the naphthaquinone compound will lend itself to therapeutic application is doubtful, as the results obtained with intravenous infusions point only to a partial synthesis at best, and the conditions cannot be assumed to be the same as those that would follow the ingestion of the substances in question. I have not been able to diminish distinctly the indican yielded by the urines of experimental animals by administering a sulphonate by the mouth, but this may be partly owing to the fact that a portion of the indol absorbed from the gut is converted by the intestinal epithelium into indoxyl and then paired with sulphuric acid. Experiments designed to compel the union of indol with a sulphonate in the gut have not yet been made, but will be undertaken. B-naphthaquinone, a substance very slightly soluble in water, reacts very slowly with indol and may possibly have advantages as an intestinal detoxicating agent.

ON THE BEHAVIOR OF SOME PRODUCTS OF THE NAPHTHAQUINONE SULPHONATES WITH DIMETHYLPARAPHENYLEN-DIAMINETHIOSULPHONIC ACID.

When the naphthaquinone sodium-disulphonate is brought into relation with a solution of dimethylparaphenylendiaminethiosulphonic acid in equi-molecular quantities, there occurs, under suitable conditions, a synthesis, which results in a violet dye possessing the following constitution.



which is easily converted into a thiazine derivative



with the elimination of sulphurous acid. This dyestuff, which is known to the trade as indochromogen S, forms a readily soluble alkali salt, a solution of which possesses only a moderate grade of toxicity for rabbits, intravenously infused. Thus from 50 to 80 cubic centimetres of a 2 % solution may be infused intravenously at the rate of 2 cc. per minute, usually without bringing about death during the infusion. If we examine an animal which has been infused in this manner with a solution of indochromogen S, the skin and connective tissues will be found to be colored very deep blue, the cartilages feebly colored, and the muscles green. The pancreas, the salivary glands, the fat, and the nervous system remain uncolored after moderate sized injections; but if the infusion be larger the gray substance of the brain is colored a dirty violet. A highly interesting feature, and one accidentally

observed in Professor Ehrlich's laboratory, is the complete filling of the system of bile capillaries in the liver, which is easily demonstrable in frozen sections.

A noteworthy feature of this injection is that it involves only the capillaries of the biliary system, which are almost uniformly distended, and thus give rise to a strikingly fine histological picture. These easily prepared pictures are, I think, superior to any staining of the bile capillaries by methods now in vogue, and demonstrate without difficulty the intracellular terminations of these vessels. Dr. W. R. Williams has, at my suggestion, worked with this method in my laboratory on the livers of animals which have been poisoned with phosphorus, with iodide of potassium and with toluylendiamine (1:3:4), with a view of studying the biliary capillaries in these conditions of poisoning. In normal animals subjected to infusions of indochromogen the bile and connective-tissues are stained blue, notwithstanding the violet color of the introduced dye. This blue dye, as it occurs in the bile, can be shown to differ in its chemical character from the indochromogen. In the experiments upon animals poisoned with toluylendiamine and subsequently infused with the indochromogen solution, the blue dye failed to find its way into the bile and the bile capillaries in the liver were only partially filled. A fuller report on the behavior of the indochromogen under pathological conditions will be given elsewhere.

If we bring a solution of dimethylparaphenylendiaminethiosulphonic acid into relation with the naphthaquinone sodium-monosulphonate instead of the disulphonate, we obtain a dye which differs from indochromogen S in several respects, and affords an instructive example of the influence of chemical constitution upon the distribution of organic substances in the living organism.

This dye has a purple-violet color, is less soluble than the indochromogen, and is considerably more toxic. If infused into living rabbits this coloring matter gives rise to a wholly different picture from that obtained from the indochromogen. The connective-tissues are colored faintly violet; the fat and the gray substance of the brain are dyed respectively purple-red and deep

purple. The pancreas, which in the indochromogen experiments was uncolored, is here dyed purple. This behavior in the organism can only be referred to the influence of the elimination of that second sulphonic acid radical, which, in the case of the indochromogen, is located in the second naphthaline nucleus. The acid character of the indochromogen was destroyed through the loss of this second sulphonic acid group, and the dye acquired certain characters that pertain to basic dyestuffs. Among these is the property to enter readily into the fat tissue and the gray substance of the central nervous system. This property is one which, as Professor Ehrlich first indicated, is apt to pertain to those basic dyes which readily diffuse into ether.

Although no thorough study of the toxicology of the naphthaquinone sulphonates has yet been made, it is certain that these bodies do not belong in the category of extremely poisonous substances. Thus a dose of 1 gram of the monosulphonate by mouth, while usually terminating fatally in a rabbit weighing 1500 grams, does not invariably produce death. A dose of this size is always followed by some prostration, diarrhœa, and frequent micturition. The urine contains both reduced and unreduced naphthaquinone sulphonate and sometimes hæmoglobin. The infusion of 0.1 gram intravenously in the course of thirty minutes is generally followed by death.

Dr. Park has very kindly investigated the bactericidal activity of the monosulphonate. The results are indicated in the following statement:

BACTERICIDAL STRENGTH OF NAPHTHAQUINONE MONOSULPHONATE IN WATERY SOLUTION UPON TYPHOID BACILLI FROM A CULTURE WHICH HAD BEEN KEPT ON ARTIFICIAL MEDIA FOR ONE YEAR.

Typhoid bacilli from bouillon culture, 50,000 to each cc. of distilled water or distilled water with disinfectant added: $\frac{1}{500}$ cc. amount plated.

Typhoid Bacilli present originally in all approximately 50,000.	After 5 min.	10 min.	30 min.	60 min.	20 hrs.
Distilled Water	50,000	50,000	50,000	50,000	35,000
" " and .1 % N.S.	50,000	50,000	50,000	40,000	0
" " and .5 % "	30,000	10,000	500	0	0
" " and 1. % "	0	0	0	0	0
" " and 1.5 % "	0	0	0	0	0

Colon Bacilli.	After 5 min.	10 min.	30 min.
Control in .00001 cc.	52		
In .1 % N.S.	38	37	45
In .5 % "	18	3	0
In 1 % "	0	0	0

B. pyocyaneus.	After 5 min.	10 min.
Control in .00001 cc.	66	60
In .5 % N.S.	0	0
In 1.0 % "	0	0

The bactericidal strength of the substance seemed about the same upon the typhoid and colon bacilli, the results in the .1 per cent seeming a little in favor of the colon bacillus being less sensitive. The larger figures at 30 minutes were undoubtedly due simply to the irregular distribution of the colon bacilli in the fluid, and did not imply a growth. The bacillus pyocyaneus was very much more sensitive, being killed by a .1-per-cent solution in five minutes.

In addition to the various biological uses of the napthaquinone compound which have already been enumerated, there are still others, the value of which cannot now be estimated owing to inadequate experience. For example, after the use of antipyretics, such as phenacetin and acetanilid, the urine contains a substance, probably para-amidophenol, which can be detected by means of its reaction with naphthaquinone sodium-monosulphonate. The study of the reactions of the urine with this substance under pathological conditions, and after the use of drugs and poisons, will doubtless in time become the subject of careful investigation, and it is possible that some medical applications will emerge from such a study.

Although it is probably no exaggeration to say that the naphthaquinone compound, which has formed the subject of discussion in these pages, is one of the most reactive color-producing substances at present known, it will not do to overlook the fact that its very lability must in itself have the drawback of depriving many of the reactions of any characteristic and specific features. In some cases we find that a whole group of compounds, like the primary aromatic amines, react in a similar manner, so that we have to deal with a class reaction for the amido group, rather than with specific reactions for individual substances. It also remains to be seen whether the usefulness of the reactions which

have been described will not suffer some restriction in those cases where we are dealing with a mixture of substances in solution, rather than solutions of chemically pure compounds. But notwithstanding these limitations, which are as yet not accurately definable, one is justified in predicting for the sulphonic acid derivatives of naphthaquinone a sphere of usefulness for the physiological chemist, as well as for the student of organic chemistry.

I wish to acknowledge the valuable help of Miss Louise M. Foster in aiding me in testing the substances mentioned in this paper, as well as very many others. To my friend, Prof. Paul Ehrlich, I feel deeply indebted for my introduction to the naphthaquinone sulphonates.

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THE INFLUENCE OF FEVER ON THE REDUCING ACTION OF THE ANIMAL ORGANISM.

By C. A. HERTER.

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THE INFLUENCE OF FEVER ON THE REDUCING ACTION OF THE ANIMAL ORGANISM.

BY C. A. HERTER.

IN a previous paper [1] I described the effects of a depression of the body-temperature on the reduction of methylene blue to leuco-methylene blue, and laid special emphasis on the impaired reducing action which is demonstrable in the muscles and gray substance of the central nervous system. Since making these observations I have carried out other experiments of a similar character upon animals in which the temperature had undergone an elevation. The outcome of these experiments appears of sufficient interest to justify me in placing on record some of the details.

The main result of the experiments which form the subject of the present paper is the demonstration of a greatly increased reducing action during fever by means of suitable color indicators (methylene blue or indophenol). The result is, as was anticipated, the opposite of that obtained by the experiments concerned with subnormal temperatures. It was found, however, that the acceleration of the reducing action which is occasioned by a rise of 3° or 4° C. gives indications of being as pronounced as the diminution in reducing action that is demonstrable by a depression of temperature through 8° or 10° C. Hence the study of cell-reductions in fever is a peculiarly satisfactory field for the application of intravital colorimetry.

The contrast between the fever animals and the controls can easily be made striking. The best results have been obtained under conditions slightly different from those recommended for the study of the effects of cold.

In most of the experiments upon the action of fever, the rabbits have been infused intravenously with a 25 per cent solution [2] of methy-

[1] HERTER: This journal, 1904. xii, p. 128.

[2] At first, the percentage of salt in the solution was 0.85 per cent, but as this concentration salts out some of the dye at low room-temperatures the sodium chloride was reduced to 0.4 per cent.

lene blue at the rate of 1 c.c. per minute, and from 50 to 75 c.c. have usually been introduced. Instead of waiting five or ten minutes after the close of the infusion before examining the organs, the animals have been killed (by ventricular incision), without delay, at the end of the infusion. These changes have been found advisable owing to the rapidity of reduction at fever heat.

Two different methods of elevating the temperature have been employed. In one set of cases the animal was enveloped in cotton batting, and an incandescent electric light was passed over the surface until the temperature of the body had been gradually elevated to the desired point, where it was without difficulty maintained during the infusion. In another set of experiments, the temperature was raised by means of hog-cholera infection. The infection was induced by means of cultures of hog-cholera of known virulence furnished me by Prof. Theobald Smith. One of the cultures sent me by Professor Smith gives rise to a temperature of 42° or 43° C. on the fourth or fifth day after inoculation with $\frac{1}{50000}$ c.c. of a twenty-four hour bouillon culture. Higher temperatures are observed later in the course of this fatal infection, but there appears to be no advantage in employing them in connection with the present investigation.

In order to illustrate the effect of elevation of temperature upon reduction in the organism, I shall give two typical protocols, one from an experiment in which the rise was caused by the external application of heat, and another in which the fever was of infectious origin.

An examination of the results recorded in Experiment 1 plainly shows the accelerating effect of elevation of temperature on the reduction of methylene blue by various types of cells. The difference in color between the corresponding parts in the two animals is the expression of the difference in reducing activity. The first evidences of this difference appeared during the infusion of the dye in the readily visible pectoral muscles, which were bared for purposes of observation. After the close of the infusion the animals were killed promptly enough to enable one to obtain an idea of the state of reduction at the end of life. The differences in color were especially striking in the brain, skeletal muscles, heart, spleen, pancreas, and liver. In all these situations reduction was more intense in the superheated animal than in the control, and, excepting the kidney, it may be stated here that this was the case not only in this experiment, but also in all the experiments of the sort that were conducted. It should be noted also that the warmed rabbit secreted less blue by

the urine and by the stomach than did his fellow, and further, that the blood in the warmed animal contained less blue than the control. These are features of interest in relation to the actual distribution of

EXPERIMENT 1.

NORMAL RABBIT (CONTROL).	SUPERHEATED RABBIT.
Temp. 38°–39° C. Weight, 1565 gms.	Temp. 42°–43° C. Weight, 1510 gms.
Received intravenously 42 c.c. of 0.25 per cent methylene blue solution in 42 minutes. Killed about 3 minutes after infusion's close.	Received intravenously 42 c.c. of 0.25 per cent methylene blue solution in 42 minutes. Killed about 3 minutes after infusion's close.
Muscles: pectorals bluish during life, quick reduction after death, the muscles of back of neck, however, remaining blued for some time.	Muscles: pectorals quite colorless during life. Muscles of back of neck completely reduced.
Application of H_2O_2 shows considerable leuco-blue in muscles.	Application of H_2O_2 shows considerable leuco-blue in muscles, more than in control muscles.
Heart found unblued (ventricles).	Heart found unblued (ventricles); contains considerable leuco-blue, more than control.
Brain: blue (turquoise).	Brain: unblued. Oxidation with H_2O_2 shows that the brain contains more leuco-blue than the control.
Spleen: blue.	Spleen: unblued; on oxidation with H_2O_2 blues to about the color of control.
Pancreas: moderately blue.	Pancreas: pale blue.
Suprarenal: colorless, does not blue on addition of H_2O_2.	Suprarenal: colorless; blues, cortex and medulla, on addition of H_2O_2.
Kidneys: blue.	Kidneys: uncolored.
Urine: scanty, deep blue.	Urine: scanty, moderately blue (greenish
Liver: purple, contains unreduced blue.	Liver: red; complete reduction of blue.
Liver pulp contains considerable paired leuco-methylene blue.	Liver pulp contains considerable paired leuco-methylene blue.
Bile: moderate amount; contains considerable methylene blue; paired leuco-methylene blue, moderate amount.	Bile: scanty; contains little methylene blue. Moderate amount of paired leuco-methylene blue (slightly more than control).
Blood: considerable methylene blue; considerable leuco-methylene blue; paired leuco-methylene blue, scanty.	Blood: little or no methylene blue; considerable leuco-methylene blue; paired leuco-methylene blue, scanty.
Stomach: much blue on surface of contents.	Stomach: little or no blue on surface of contents.

dye in the body. The criticism might be made that the differences in color in the two animals were possibly referable to variations in the distribution of the dye rather than to unequally energetic reduction. In answer to such a criticism it might be said that if one

organ or a group of organs in the warmed animal received less blue than the corresponding organ or group of organs in the control, there would necessarily (since the infusions are of the same magnitude) be a compensating difference in the opposite direction, in some other part of the body, whereas in reality the color contrasts between the organs of the two animals were so widespread that they may be designated universal. Further and more convincing evidence is, however, not wanting to show that the color differences are not dependent on unevenness of distribution. Experiments made with eosene (which undergoes no reduction or other demonstrable chemical change in the body) showed that the distribution of this dye is essentially the same in the organs of the normal rabbits as in the corresponding organs of animals in which the temperature has been raised, — the conditions of infusion having, of course, been kept the same in these experiments. Finally, the ease with which methylene blue undergoes oxidation gives us a direct method of determining the actual distribution of the dye in the organism, at least in the case of the central nervous system and the muscles. If we have any doubt whether the brain has taken up as much dye as its fellow, we have only to pour upon a freshly cut surface a solution of hydrogen peroxide, when the leuco-blue or reduced blue is rapidly oxidized to the dye itself. By means of this method, it was easy to show that the brain and muscles of the warmed animal held not less but *more* dye than the corresponding parts of the normal rabbit.

When we come to the other organs, especially the liver and kidneys, the conditions are not so simple, for they are complicated by the occurrence of a synthesis or pairing of the methylene blue with some unknown constituent of the cells. The substance thus formed I have called paired leuco-methylene blue, or more briefly "paired substance." The important characteristic of this substance in the present connection is that it fails to be oxidized (in neutral or alkaline medium) to methylene blue, and hence escapes recognition in the simple process of oxidation. The dye can, however, be unmasked by boiling the organ pulp with a dilute acid — acetic being perhaps the best for this purpose. Until I recognized the pairing process in the liver and elsewhere, it was impossible to account for the disappearance of the infused methylene blue, since it was evident that the sum of the simple leuco-blue and the unreduced blue was far from being equal to the amount of dye infused.

It would be incorrect to give the impression that the total amount

of methylene blue in the febrile organs was always or even usually in excess of the amount in the corresponding organs of the controls. Even in animals of the same temperature, infused under the same conditions, the brain and muscles (structures holding little or no

EXPERIMENT 2.

NORMAL RABBIT (CONTROL).	INFECTED RABBIT (HOG CHOLERA).
Temp. 39° C. Weight, 1450 gms.	Temp. 40.5° C. (4 days after inoculation).[1] Weight, 1435 gms.
Infused intravenously 31 c.c. 0.25 per cent methylene blue solution, 1 c.c. per minute. Killed at close of infusion.	Infused intravenously 31 c.c. 0.25 per cent methylene blue solution, 1 c.c. per minute. Death at close of infusion.
Muscles: blue during life. At death, blue was rapidly reduced. With H_2O_2 moderately blue.	Muscles: no bluing during life; with H_2O_2 less blue than control.
Heart: Ventricles blued.	Heart: ventricles unblued.
Brain: almost completely reduced; blues deeply on exposure to air; with H_2O_2 very deep blue.	Brain: completely reduced, but blues only slightly on exposure to air; with H_2O_2 blues somewhat less deeply than control.
Spleen: blue.	Spleen: red.
Pancreas: not noted.	
Suprarenals: not noted.	
Kidneys: unblued; considerable leuco-blue in cortex.	Kidneys: blue; papillae and medulla blue on exposure to air.
Urine: none.	Urine: none.
Liver: bluish; leuco-blue, considerable; pulp yields considerable paired leuco-methylene blue.	Liver: gray; contains little or no leuco-blue; nearly all blue exists as paired leuco-methylene blue; quantity about same as in control.
Bile: quantity moderate; some methylene blue; some paired methylene blue.	Bile: scanty; no methylene blue (as such); very little paired leuco-methylene blue (*i. e.* no secretion).
Blood: serum blue; very little leuco-blue; considerable paired leuco-methylene blue.	Blood: serum uncolored; little or no leuco-blue. All dye is present as paired leuco-methylene blue.
Stomach: contains considerable blue.	Stomach: contains very little blue.

[1] The temperature of this animal had been higher, but it fell when the animal was placed on the holder, as is often the case.

paired substance) often show, on oxidation with hydrogen peroxide, moderate inequalities in the content of dye. In the case of experiments made at an elevated temperature, the greater amount of dye is found sometimes in the muscles and brain of the control, sometimes in the structures of the superheated mate. The evidence of increased reduction is, of course, especially pronounced in those cases where the

brain and muscles not merely are colorless, but contain more total dye-stuff than their fellow-organs.

In essential respects the conditions in Experiment 2 after infusion resembled those found in Experiment 1; that is to say, the various parts, including brain, muscles, heart, spleen, and liver, showed the presence of less blue in the febrile rabbit than in his fellow. The differences were, however, less pronounced than in Experiment 1, and this is hardly surprising, inasmuch as the disparity in temperature was only 2.5° C.; whereas, in Experiment 1 the inequality amounted to probably not less than 4° C. In other observations in animals infected with hog cholera, higher temperatures were obtained, and in such instances the results were indistinguishable from those seen in animals whose temperature had been raised to an equally high level by external application of heat.

There is usually a distinct difference between the color of the blood of the febrile animal and that of the control, the former containing less methylene blue as such and more leuco-methylene blue. The bile in the gall-bladder of the febrile animal is almost always scanty, and contains less methylene blue than the bile from the control. The stomach also shows a diminished secretory activity when the temperature is elevated, for little or no blue finds its way into the interior of this organ, whereas in the control animals the food is found covered by a layer of mucus mixed with unreduced blue. Once, however, I found these conditions reversed. As regards the quantity and character of the urine secreted during the infusion the results are extremely variable. I have generally found that the febrile urine contains rather less methylene blue and more leuco-methylene blue than the urine from the normal control, but there may be more blue in the febrile urine. It seems probable that the wide variability noted in wholly normal animals with the same temperature affords a sufficient explanation of these irregularities. Occasionally, as in Experiment 2, there is no secretion during the infusion. In general, it can be stated that the quantity of dye recovered in the urine is too small to exert a material influence on the quantity or distribution of that which remains in the organism.

The observation that methylene blue is capable of serving as an admirable indicator of the acceleration of reduction resulting from an elevation in temperature naturally suggested that the various kinds of cells in which the reducing powers had been watched during life might be advantageously studied in vitro in respect to this activ-

ity. The prospect of being able to study individually the properties of different tissues, unhampered by the uncontrollable and unmeasurable interactions characteristic of the living organism in its entirety, made it appear worth while to seek a method of conducting such experiments under conditions permitting a measurement of the processes in question.

After some unsuccessful trials, the following method of measuring approximately the reaction velocity of reduction was adopted. The tissues to be studied (liver, kidney, muscles, etc.) were taken from a dog or rabbit (which had been bled) and subdivided in an ordinary meat machine. A finer degree of subdivision was secured by trituration with fine sand. Definite weights of tissue thus prepared and mixed with sand were placed in thin walled capacious test-tubes of hard glass, one and one-quarter inches in diameter, to which a fixed volume of distilled or tap water was added. For example, to 2 or 4 gms. of triturated liver was added usually 25 gms. of water. To this mixture was added at the proper time 1 c.c. of a weak methylene blue solution (0.025 per cent in distilled water) at the proper temperature. In order to secure better contact of the dye with the particles of tissue, a constant stream of washed and neutralized nitrous oxide gas was passed through the mixture.[1] As our gas liberated a very slight quantity of oxygen, its action, aside from a mechanical one, must have been to slightly retard reduction. Carbon dioxide was abandoned because of the disturbing effect exerted by its acid properties.

The temperature within the tubes was easily regulated, and there was no difficulty in keeping their contents in a practically anaërobic state. In order to insure the rapid and thorough mingling of the dye with the remaining contents of the tube, it has been found helpful to inject the methylene blue directly into the rubber delivery tube through which the gas enters the lowest part of the tube. The reduction tube is prepared for action by allowing the nitrous oxide to bubble actively through it for five minutes before the dye is introduced.

As regards the endpoint of the reaction, it must be said that this is not always as sharp as could be desired, and that it seldom happens

[1] A drawing of the apparatus will be given in a subsequent paper. Each tube is fitted with a rubber cork with three openings, one for the tube delivering the gas, one for a thermometer, and a third for the exit of the gas. Two tubes are generally operated simultaneously. The desired temperature within the tubes is secured by their immersion in a beaker of water.

that the original color of the tube is regained, even through prolonged action of the tissues on the blue. For this reason, it has been found best to take the reading when the last trace of the green-blue disappears from the mixture in the tube. Where control observations are being made, it is usually not difficult to fix upon an arbitrary endpoint which is the same for both tubes.

By means of the method thus indicated, it has been possible to study the influence of many conditions and substances upon the velocity of reduction, including the action of acids and alkalies, the ions of neutral salts, the effects of colloidal solutions,[1] etc. At present reference will be made only to a few typical observations on the influence of temperature.

The following are a few examples of the influence of temperature and mass of tissue (rabbit's liver):

1 gm. liver + 1 c.c. blue solution + 25 c.c. H_2O at 38° C. reduced in 24¾ minutes.
1 gm. liver + 1 c.c. blue solution + 25 c.c. H_2O at 43° C. reduced in 18 minutes.
2 gms. liver + 1 c.c. blue solution + 25 c.c H_2O at 38° C. reduced in 3½ minutes.
2 gms. liver + 1 c c. blue solution + 25 c.c. H_2O at 43° C. reduced in 2 minutes.

Material from the liver of another rabbit gave the following results:

1 gm. liver + 1 c.c. blue solution + 25 c.c. H_2O at 38° C. reduced in 8 minutes.
1 gm. liver + 1 c.c. blue solution + 24 c.c. H_2O + 1 c.c. 0.85 per cent NaCl solution at 38° C. reduced in 8 minutes.
1 gm. liver + 1 c.c. blue solution + 25 c.c. H_2O at 43° C. reduced in 4 minutes.
1 gm. liver + 1 c.c. blue solution + 24 c.c. H_2O + 1 c.c. 0.85 per cent NaCl solution at 43° C. reduced in 5 minutes.
2 gms. liver + 1 c.c. blue solution + 25 c.c. H_2O at 38° C. reduced in 1¼ minutes.
2 gms. liver + 1 c.c. blue solution + 25 c.c. H_2O at 43° C. reduced in 45 seconds.

With larger quantities of triturated tissue, say 4 or 5 grams of liver, reduction may occur so rapidly that it is difficult to measure the difference in velocity at 38° and 43° C.

At one time it seemed to me that our experiments indicated a greater reaction velocity than is characteristic of simple chemical

[1] An interesting field for observation is a comparison of the reducing action of different structures in the same organism, and of the corresponding structures in different groups of animals. In the present experiments the great activity of liver and kidney as compared with brain and muscle is an obtrusive feature. The superiority of the dog's liver over the liver of the rabbit in regard to the power of reduction can be easily shown. The action of poisons has not yet been taken up with this method, except to a limited extent.

reactions; but at present it is doubtful if that view can be maintained. In order to gain information as to the influence of temperature on the velocity of reduction in the absence of organized material, the tubes were filled with a solution of 10 c.c. of (approximately) 3.71 per cent glucose solution, 1 c.c. methylene blue solution, 2 c.c. $\frac{n}{10}$ sodium hydrate solution, and 12 c.c. water. At 38° C., reduction occurred in nine and one-quarter minutes; at 43° C. in six minutes; at 48° C. in four minutes. The results thus derived are apparently of the same order as those obtained with living tissues.

It is clear that many factors influence the velocity of reduction by living animal tissues. In some of the above experiments the effect of a small amount of sodium chloride is seen,[1] and it can be shown that there is a high degree of sensitiveness to the presence of hydrogen and hydroxyl ions. The differing activity of the livers of different individuals in vitro is a striking thing, and reminds one of the individual differences noted in the course of intravital infusions. A factor which may become disturbing in test-tube experiments is the postmortal decline in activity of the cells. In the case of muscle, especially rabbit muscle, this is rapid. The liver, however, usually retains a high grade of reducing activity for several hours after death.

It is believed by the writer that the demonstration of the action of fever on the organism by the intravital method described in these pages will not only prove serviceable in the study of the pathology of fever, but will afford a highly instructive object lesson in class work. Probably the method of studying reduction in vitro will prove of considerable use in the analysis of the many factors that affect the process of reduction. I hope before long to report on the behavior of different kinds of living tissue under physiological and pathological conditions.

I wish to acknowledge the valuable assistance I have received from my laboratory assistant, Mr. Edward O'Brien, in the conduct of these experiments.

[1] At 38° C. sodium chloride, in larger amount than in the experiment given above, was found to accelerate reduction, but at higher temperatures it was frequently observed to exert the opposite influence.

THE LATE DR. GEORGE A. SPALDING.

BY

DR. C. A. HERTER

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In Memorium.

The Late Dr. George A. Spalding.—Through the premature death of George Atherton Spalding the medical profession of this country has lost one of its most alert and discerning practitioners and the community a character of rare force and gentleness, to whom every worthy social aim made a powerful appeal. The end came almost without warning, during the afternoon of October 2nd, from an unsuspected affection of the heart and apparently in consequence of excessive exertion in the course of professional duty. Dr. Spalding was born on January 14, 1849, in Kentucky. He sprang from a long line of educated ancestors, many of whom attained distinction as medical men. His grandfather studied medicine at Guy's Hospital and brought over to this country from the celebrated Dr. Jenner the first vaccine against smallpox, which he delivered to Dr. Waterhouse. After an academic course at Yale University, the late Dr. Spalding obtained his medical education at the College of Physicians and Surgeons, where he acted during two years as assistant to the eminent physiological teacher and investigator, Professor John C. Dalton. The close contact with an acute and cultivated mind, used to deal with medical problems from the physiological standpoint, was of inestimable value to the young student of medicine and accounts for the active interest in the scientific basis of medicine which was shown by Spalding throughout his long and successful career. After graduation at the medical school, he served as an interne at St. Luke's Hospital (1875-'6), where he quickly won the confidence and admiration of his colleagues. Thus equipped for work, Spalding began life in New York as a general medical practitioner. He soon built up a large practice and developed the exceptional gifts which made him one of the most competent physicians in the great city. These gifts were both intellectual and moral. Perhaps Spalding's most distinguishing

trait was quickness of insight into complex and difficult medical and human situations, calling for prompt and individual treatment. He had the intelligence to promptly apprehend what should be done and the force to do what he deemed right. His patients either obeyed him implicitly or were requested to seek another adviser. People soon learned to trust the mind and heart of the clear eyed, earnest man, whose first interest was to do his duty, promptly and fully, without sparing himself. This practice grew apace until it made heavy demands on thought and strength. To meet these demands while ever striving to elevate the quality in his work, Spalding was compelled to sacrifice his personal culture in many directions and to live a life of great abstemiousness and self control. The courage with which he made personal sacrifices year after year for the sake of his work will never be known beyond the small circle of those with whom he was intimate, for he bore much in silence. Notwithstanding the ever increasing exactions of private practice, Spalding made time for outside professional work designed to increase his efficiency as a physician. From 1879 to 1894 he served as an attending physician at the House of Refuge on Randall's Island, where his energy and upright methods proved invaluable to the public service. In 1896 he was appointed to the important position of attending physician to St. Luke's Hospital, the active duties of which brought him great satisfaction, although he had many regrets that he could not give so much of his time to these duties as he would have wished. This position he held at the time of his death. Few general practitioners have kept in such close personal touch with the most progressive members of the profession. Spalding had the intuitive feeling for the qualities of people which comes only to sensitive and refined natures. He enjoyed mixing freely among men, and his personal charm made him *persona grata* in the societies frequented by the most distinguished members of the medical profession. This contact had a highly important practical influence on his efficiency in private and hospital work. He learned to know well the professional and personal qualities of the men who could help him in emergencies and

exceptional cases. He was in a position, therefore, to give his patients the benefit of the best available medical and surgical skill. This he did with the utmost freedom and with highly refined judgment. Spalding was not a teacher of students, nor did he publish often, but he possessed the insight which, under conditions of greater leisure, would have brought success as a teacher and the judgment to enrich literature with well recorded cases. Circumstances limited his talents and activities to the practical duties of his profession. In the skilful discharge of these humanitarian duties he was actuated by the highest and broadest motives. He sought not merely to give his patients physical security and comfort, but to educate them to conform to the ways of Nature through the exercise of intelligent self control. He studied to prevent disease as well as to cure it, and perhaps few who have dealt with individuals rather than with masses have better succeeded in this. Thus his influence on the community was widespread for good and hardly measurable by common standards.

THE PRODUCTION OF METHYL MERCAPTAN BY FECAL BACTERIA GROWN ON A PEPTONE MEDIUM.

BY

C. A. HERTER.

FROM
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THE PRODUCTION OF METHYL MERCAPTAN BY FECAL BACTERIA GROWN ON A PEPTONE MEDIUM.

By C. A. HERTER.

(Received for publication, January, 22, 1906.)

It is surprising that almost no systematic study has been devoted to methyl mercaptan, a common product of the putrefactive cleavage of proteid, in its relation to intestinal putrefaction. Nencki,[1] who discovered this substance among the products of putrefaction, obtained a small amount of it from a large quantity of human feces, and reached the conclusion that it is a usual product of intestinal putrefaction. This conclusion is probably correct, although, strictly speaking, it does not appear fully justified by Nencki's observations, for the reason that the material used by him could not have been sufficiently fresh to exclude mercaptan formation during a period of exposure to the air. Working with smaller quantities of material than were employed by Nencki, I have never been able to obtain more than a trace of mercaptan (probably the methyl compound), and have usually been able to obtain no evidence whatever of its presence in fresh human material, whether from presumably normal persons or from such as were suffering from intestinal disturbance. But the failure to find mercaptan in the contents of the lower bowel does not prove that this substance has not been formed, for it is possible and even likely that there is some mercaptan production in higher portions of the large intestine, where it is certain that the gas would be readily absorbed.

As it appeared that something of interest might be learned from the study of the ability of fecal bacteria from various sources to form mercaptan when growing on a medium which does not readily yield this compound, a considerable number of experiments were made with suspensions of the mixed fecal bacteria from different persons. After growing the bacteria in a two per cent. peptone solution for twenty-four hours at the temperature of

[1] *Monatsh. f. Chem.*, x, p. 862, 1889. Das Methylmercaptan als Bestandtheil der menschlichen Darmgase.

37° C., the entire culture (100 c. c.) was in each case transferred to a flask communicating through a calcium chloride tube with an Erlenmeyer flask containing isatin dissolved in concentrated sulphuric acid. A current of air was then drawn through both flasks so that any mercaptan given off from the culture flask would enter the isatin-sulphuric-acid flask. The presence of mercaptan is indicated under these conditions by a gradual change of the isatin solution from red to olive-green or grass-green.[1] The method is not adapted for quantitative determinations but some idea can be gained through it of the quantity of mercaptan present in a culture, and it further serves to indicate differences in the amount formed in different cultures. Twenty-five milligrams of a one per cent. solution of methyl mercaptan suffice to gradually alter the red isatin solution (about 50 c. c.) to a deep green in the course of ten minutes. Reactions as strong as this are occasionally obtained from one hundred cubic centimeters of a bacterial culture, but quicker reactions of this intensity have not been found.

With the aid of the method[2] here outlined, more than one hundred and thirty observations have now been made on aërobic cultures of mixed fecal bacteria.[3] A fact which stands out clearly as a result of these observations is that the bacteria derived from normal persons (*i. e.*, showing not more than moderate quantities of putrefactive derivatives in the urine and otherwise in good health) do not usually yield more than a trace of mercaptan when grown for twenty-four hours on a two per cent. peptone solution. In a number of instances, a pronounced trace of mercaptan has been obtained after prolonged aspiration through the apparatus mentioned above. In a few instances, a moderate reaction has been obtained after aspiration for ten or fifteen minutes, and such a reaction

[1] This method has been used by Niemann, *Arch. f. Hyg.*, xix, p. 126, 1893; also by Bauer, *Zeitschr. f. physiol. Chem.*, xxxv, p. 346, 1902.

[2] The method was in many instances supplemented by the use of the mercuric cyanide method.

[3] The organisms were grown in 250 c. c. flasks and the volume of the peptone solution was 100 c. c. The upper portion of the culture was under aërobic conditions, the lower part, under anaërobic conditions.

has sometimes been repeatedly obtained from bacteria from the same person. Strong reactions were several times obtained from the organisms obtained from breast-fed babies apparently in good health, and once a strong mercaptan reaction was noted in the case of a growth made from material from a young man in good health but troubled with constipation With these exceptions, I have noted strong mercaptan production only in cultures made from material derived from pathological sources. The strongest reactions were obtained from bacteria derived from persons suffering from pernicious anæmia, depressive mental st: tes, infantile marasmus, fat diarrhœa, and cases of chronic intestinal indigestion (in children) characterized by abdominal distension, anæmia, and retarded development. In the cases referred to, mercaptan production has usually been a persistent rather than a transitory manifestation. In two cases of pernicious anæmia in which rapid improvement occurred in association with rest in bed and care in diet, the fecal bacteria ceased to produce mercaptan, coincidently with this improvement.

Thus the facts at present at our disposal indicate that the pronounced formation of methyl mercaptan by fecal bacteria growing on peptone solution is commonly a manifestation of pathological rather than normal bacterial activity, although it is doubtful whether its occasional production by micro-organisms from a human individual is to be regarded as necessarily unphysiological. Especially in the case of young children in good health is a moderate mercaptan a common occurrence. This conclusion is the more noteworthy because it is entirely different from the conclusion reached from a study of hydrogen sulphide production under similar condition. In the case of hydrogen sulphide formation, it may be said that this sulphur compound has almost regularly been obtained by growing fecal bacteria on a peptone medium, and that it is by no means uncommon to find an abundant hydrogen sulphide production where no trace of mercaptan is obtainable.[1]

We have as yet little accurate information as to the conditions under which methyl mercaptan is produced in the course

[1] The bacteria from certain acid stools from children may fail to make hydrogen sulphide, although under anaërobic conditions mercaptan may be produced.

of bacterial activity. Nencki regarded it as a result of anaërobic decomposition, but it appears that, although this view is for the most part correct, anaërobic conditions are not always essential to its formation. The bacteria from the feces of a patient with pernicious anæmia grown on a peptone solution under aërobic conditions gave much hydrogen sulphide and no mercaptan; when the organisms were grown anaërobically (under carbon dioxide) there was an abundant production of mercaptan (presumably the methyl compound) in addition to hydrogen sulphide. On a medium containing asparagin, ammonium lactate, alanin, glycocoll, cystin, and salts, I obtained a very abundant production of hydrogen sulphide from fecal bacteria, but not a trace of mercaptan.

Pure cultures of the aërobic bacteria derived from feces capable of inducing mercaptan formation have failed to give this gas. B. coli communis growing on the peptone solution gives hydrogen sulphide but no mercaptan. B. putrificus I have not yet tried in pure culture, but Dr. Rettger tells me that it early produces mercaptan when grown on an egg-meat medium.

Although it is not at present clear what inferences may be safely made, from the formation of mercaptan by the fecal bacteria, with regard to mercaptan production and absorption in the intestine[1] from which the bacteria were derived, it seems clear that further observations are desirable along lines suggested by the results embodied in this paper. It appears also desirable that a careful pharmacological study of the prolonged action of methyl mercaptan should be undertaken, and such a study is now under way in my laboratory.

[1] Some observations which I have made upon bacteria found at early autopsies in various parts of the intestinal tract show that mercaptan-producing organisms may be present in the upper part of the ileum. Thus from an autopsy on a child of fifteen months, dead of pneumonia, the mercaptan production by the bacteria of the ileum was even more pronounced than that of bacteria obtained from lower levels. In the case of a child dead of marasmus, the stomach contained bacteria which made hydrogen sulphide abundantly and some mercaptan. The bacteria of the duodenum and jejunum made neither of these gases, but those of the ileum produced them both in abundance.

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ON THE SEPARATION OF INDOL FROM SKATOL AND THEIR QUANTITATIVE DETERMINATION.

BY

C. A. HERTER AND M. LOUISE FOSTER.

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ON THE SEPARATION OF INDOL FROM SKATOL AND THEIR QUANTITATIVE DETERMINATION.

BY C. A. HERTER AND M. LOUISE FOSTER.

(Received for publication, July 10, 1906.)

In a recent publication[1] it was pointed out that the presence of indol in a solution can be detected by means of β-napthaquinone-sodium-monosulphonate, and that owing to certain properties possessed by the compound formed by the union of indol with this napthaquinone compound it is possible to determine with considerable accuracy the quantity of indol in solution. As indol is sometimes associated in the course of putrefaction with skatol (this is not infrequently the case with the contents of the large intestine) it becomes desirable to have a method for the separation of indol from skatol. These putrefactive decomposition products may be separated by means of their picrates, but the method involves so much time as to make it unfit either for clinical or ordinary chemical investigations. It is believed that the method about to be described will prove practically helpful in effecting the rapid and nearly complete separation of indol from skatol and that it will further serve for the determination of the quantity of skatol present.

The method is based on the fact that by means of the napthaquinone compound above mentioned, it is possible to remove the indol almost completely from a solution containing both indol and skatol, and that the skatol remaining after the removal of the indol can be distilled and recognized by means of the dimethylamidobenzaldehyde reaction described by Ehrlich.

If one takes a putrefactive mixture containing both indol and skatol, these bases should first be distilled either in acid or alkaline solution, sometimes preferably with the aid of steam. In the distillate the skatol passes over earlier than the indol, as can be easily shown by means of the blue color which it gives on boiling with Ehrlich's aldehyde. To the distillate containing

[1] "A Method for the Quantitative Determination of Indol." This Journal, i, p. 257, 1906.

the indol enough sodium or potassium hydroxide is added to render it slightly alkaline. An excess of the β-napthaquinone-sodium-monosulphonate is now added to this solution. This substance in the course of a few minutes reacts almost completely with the indol present but not with the skatol, and the result of the reaction is the appearance of the blue or purplish-blue precipitate of the newly formed indol naphthaquinone compound, which may be removed by filtration. In cases where the concentration of indol is too small to give rise to a precipitate when treated with the napthaquinone compound the solution simply develops a green or greenish-blue color. The solution is now acidified and subjected to distillation (with or without the use of steam). The skatol passes over into the distillate; the indol is held back in the form of the napthaquinone compound with the exception of a very small uncombined portion which passes over with the skatol. The amount of indol, however, which passes over after treatment with naphthaquinone is so small that it is practically negligible, although its presence is detectable through the red color which it gives when acted upon by dimethylamidobenzaldehyde. The distillate containing skatol is boiled with a solution of Ehrlich's aldehyde in sulphuric acid.[1] A slight amount of dilute hydrochloric acid is now added and has the effect of intensifying the blue color produced by boiling the skatol with the Ehrlich aldehyde solution. A little experience is required to find the amount of hydrochloric acid which gives the maximal intensification of the reaction. An excess of hydrochloric acid causes the blue color to fade. It is important to use an excess of the Ehrlich aldehyde solution in order to develop fully the color reaction with skatol. The color obtained through the action of Ehrlich's aldehyde upon skatol is purple-blue rather than blue so long as the solution is hot. On cooling it under the tap the blue color asserts itself more strongly and the solution may become somewhat opalescent from the separation of uncombined dimethylamidobenzaldehyde. Chloroform is now added to the solution containing the blue product. On agitation with the solution this carries out the blue color, and the chloroform assumes a pure blue tint. By means of a good colorimeter the quantity

[1] Five per cent. aldehyde in ten per cent. sulphuric acid.

of skatol present in the original solution may be approximated by the intensity of the color reaction.

On evaporating the chloroform containing the blue color resulting from the action of dimethylamidobenzaldehyde on skatol one obtains an amorphous blue material which can be partially purified from the admixture with Ehrlich's aldehyde by the use of petroleum ether. The nature of this compound is not at present known. The melting-point of our preparation lay between 65° C. and 66° C.

Apparently few micro-organisms growing on bouillon are capable of making skatol, at least within a week or ten days, although many bacteria are able in the same length of time to produce considerable indol. It was found that by inoculating an asparagus-bouillon medium with mixed flora from the feces of a normal pig or from certain human fecal material considerable skatol was formed in the course of two weeks.[1] From a putrefactive mixture obtained by the action of pig's feces, 17.29 milligrams[2] of skatol were recovered from 244 cubic centimeters of the asparagus-bouillon medium—a very large yield of skatol. The separation of skatol from the culture was in this case easily effected by means of the method outlined, and the blue compound obtained in chloroform solution was indistinguishable from that obtained from a pure solution of skatol.

In another instance in which indol was present in a culture containing a larger quantity of skatol than indol, an equally satisfactory separation was made.

The method of separating indol and skatol here described has been used in a routine way during the past eight months in connection with the study of the feces and has given satisfaction. To twenty-five grams of the material has been added twenty cubic centimeters of water and one to two cubic centimeters of a ten per cent. sodium hydroxide solution. The suspension is then subjected to distillation with the aid of steam until the distillate no longer gives a color reaction when boiled with the

[1] The exact conditions that must prevail to secure this large formation of skatol have not been determined and difficulty has been met with in duplicating these results at will.

[2] This result was reached by a colorimetric observation. Other observations on the same material gave the following fairly concordant results: 17.87 mg., 18.62 mg., 18.62 mg., 19.29 mg.

dimethylamidobenzaldehyde solution. Ordinarily the indol and skatol present go over completely within an hour, but where the material develops frothing a more prolonged distillation may be necessary. When the distillate has been obtained, it is treated in the mannner above outlined for the separation of indol and skatol. In some cases one obtains only skatol from the feces, but as a rule indol is also present. In the presence of indol the chloroform extract, instead of being a pure blue, may have a slightly purplish tinge, owing to unavoidable admixture with a slight amount of indol.

It is important in making the colorimetric estimations of the quantity of skatol present to employ a standard color solution for comparison with the color obtained from the distillate containing the skatol. Various dyes have been tried with a view to obtaining a standard solution which will retain its color unchanged. Experience has shown that the best standard color solution is one obtained from a solution of skatol. Although such a solution fades after a few days, especially when exposed to the light, and may assume a greenish tint, in the dark it may last several weeks without undergoing appreciable change. Moreover as the skatol standard solution is readily prepared, there is little disadvantage from being compelled to renew the color solution from time to time. This solution may be conveniently prepared by dissolving five milligrams of skatol in water and acting upon it with an excess of dimethylamidobenzaldehyde. It commonly requires from one hundred to one hundred and fifty cubic centimeters of chloroform to extract completely the blue coloring matter which has already been described. The quantity of coloring matter present is sufficient to impart a deep blue color to this volume of chloroform. Ten cubic centimeters of this solution are placed in the receptacle of the Duboscq colorimeter and used as a standard for comparison with the chloroform color solution obtained from the distillate to be tested. The matching of the colors can usually be made very closely. In cases where the quantity of skatol is so small that the trace of indol present influences the color of the chloroform solution, changing it to violet or even purple, it is more difficult to obtain a satisfactory matching of colors. In this case it may be necessary to add a small quantity of indol to

the skatol employed in making the standard color solution. This then imparts to the standard color solution a violet tint like that obtained from the distillate to be matched. It seems unnecessary to give further details. After some experience with the method of matching colors it is possible to employ the method so that it will give satisfactory quantitative results.

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A METHOD FOR THE QUANTITATIVE DETERMINATION OF INDOL.

BY

C. A. HERTER

AND

M. LOUISE FOSTER.

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A METHOD FOR THE QUANTITATIVE DETERMINATION OF INDOL.

BY C. A. HERTER AND M. LOUISE FOSTER.

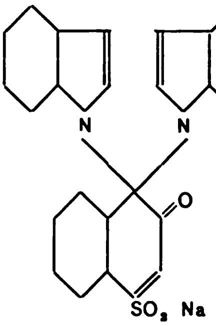
(Received for publication, November 20, 1905.)

The condensation of indol with β-naphthaquinone-sodium-monosulphonate, a color-reaction at once sensitive and striking, has been used by us as the basis of a method for the quantitative estimation of indol. In a previous paper [1] by one of us, a description of this reaction is given. A dilute solution of indol (say 1:100,000 parts water), made slightly alkaline with potassium hydroxide, gives with one drop of 2 per cent. napthaquinone-sodium-monosulphonate a blue or green-blue color. This color is varied by excess of either reagent and by heat. If to a warm, more concentrated solution of indol the alkali is added and then the napthaquinone compound, a dark precipitate is formed, which upon examination will be found to consist of well-defined acicular needles, bluish in color, and closely felted together. This compound is very slightly soluble in water, more soluble in alkali, and moderately soluble in chloroform, with the production of a red color.

Analysis to determine the nitrogen content of this substance showed the presence of two nitrogen atoms in the molecule, indicating that the compound is derived from two molecules of indol, while the determination of the sulphur content made it evident, first, that the sulphonic acid group is retained, and secondly, that but a single molecule of β-napthaquinone-sodium-monosulphonate enters into the combination. The condensation probably takes place, therefore, between one of the carbonyl groups of the napthaquinone compound and the imide groups of the indol molecules, with the elimination of water. It is probable that the indol substituents occupy the para position to the sulphonic acid group, and that the constitution of the

[1] *Jour. of Exper. Med.*, vii, No. 1, 1905.

compound, di-indyl-di-hydronaphthaline-keto-sodium-monosulphonate may be represented thus:



It must be admitted, however, that other constitutions for this compound are imaginable, and it must not be forgotten that the pyrrol ring may be broken at the double bond.

A slight blue precipitate forms in solutions containing one part of indol to 256,000 parts of water; in greater dilution the coloration is green, and fails entirely when the dilution is 1:1,024,000 parts. Chloroform indicates the presence of di-indyl-di-hydronaphthaline-keto-sodium-monosulphonate by its faintly pink color, even in this extreme dilution. The sensitiveness of the di-indyl-di-hydronaphthaline-keto-mono-sulphonate reaction, the insolubility of the newly formed compound in water, and the thorough extracting power of chloroform suggested the possibility of a quantitative method for the determination of indol and experiments were made with this idea in view.

Some carefully prepared di-indyl-di-hydronaphthaline-keto-sodium-monosulphonate was washed and dried to constant weight, placed in a separatory funnel in which was a small quantity of water, and extracted with chloroform in small portions until the chloroform showed no coloration. The combined portions of chloroform were then distilled, the residue transferred to a tared watch-glass, the last traces of chloroform allowed to evaporate, and the weight obtained. It was found that essentially the entire quantity of the di-indyl compound could be recovered.

The condensation of indol with the naphthaquinone com-

pound requires even in dilute solutions considerable time for its completion, and it has been found desirable to allow a period of not less than ten minutes to elapse before shaking out the condensation product by means of chloroform. If the product be shaken out with chloroform before the reaction has been completed, the uncombined indol passes into the chloroform and a loss is thus occasioned. It is also important to add a slight excess of the naphthaquinone compound in order to secure the condensation of all the indol present. The naphthaquinone solution should be added in an amount sufficient to give the solution a distinctly yellow tinge after the condensation product has been shaken out with chloroform, this tinge being an evidence of the presence of the naphthaquinone compound in excess.

Another experiment was performed with the components of the above compound. A weighed portion of indol, 0.02 gram, was transferred to a separatory funnel and dissolved in the smallest quantity of water possible, made alkaline with potassium hydroxide, and the naphthaquinone compound added in slight excess. The characteristic precipitate which formed was then shaken with chloroform until the latter remained uncolored, the chloroform distilled, and the residue cooled and weighed. Of the original indol, 96.5 per cent. were recovered. Further experiments were made to ascertain whether the indol could be recovered from a solution containing a proteid. A 0.3 per cent. bouillon, made from beef extract and Witte's peptone, was selected for this purpose. A weighed portion (0.02 gram) of indol was dissolved in the smallest quantity of water and added to about 150 c.c. of the bouillon and the solution made slightly alkaline and distilled. The last portion of the distillate was tested and found still to contain traces of indol; 100 c.c. of water were added and the process continued until negative results were obtained on testing a few drops of the distillate with both the naphthaquinone reagent and with para-dimethylamidobenzaldehyde. The distillate was then made alkaline, the indol precipitated with β-naphthaquinone-sodium-monosulphonate, and the di-indyl compound extracted with chloroform. Good results were obtained, even with a slightly acid bouillon.

A similar experiment was made with Dunham's peptone solution, and equally good results were obtained, 97.3 per cent. being the proportion of indol recovered.

Some observations have been made which indicate that the method here described will prove of service in determining the indol content of the feces. The material to be examined is rendered alkaline with caustic potash in order to hold back phenol. Distillation, preferably with steam, is then practiced. The distillate contains ammonia and perhaps indol and skatol. In order to remove the ammonia, which gives a green color with the naphthaquinone compound, it is necessary to acidify the distillate and distill again. This distillate contains the indol and skatol. The indol is separated from the solution by adding to it an excess of the naphthaquinone compound and then alkalizing with caustic potash. Under these conditions the blue condensation product of indol and naphthaquinone is gradually formed and can be almost completely shaken out by chloroform. A large excess of the naphthaquinone compound should be avoided, as this substance passes to a slight extent into the chloroform and may thus slightly modify the red color imparted to it by the indol compound.

It has been easy to show by means of this method that there is a rough parallelism between the intensity of the indican reaction of the urine and the quantity of indol in the feces.

If the quantity of indol to be determined is very small (say less than 0.25 mg.), it is necessary to concentrate the red compound by evaporation, and in any case one should employ for the colorimetric determination of indol a distinctly pink solution of the di-indyl compound in chloroform. The quantity of indol present may now be estimated colorimetrically, preferably by means of the Duboscq colorimeter. It has been found that a solution of Casella & Co.'s brilliant cochineal can be used for comparison, as it gives tints closely resembling those of the indol compound in chloroform, but it is preferable to use as a standard of comparison a chloroform solution of the indol condensation product kept for this purpose or made up freshly. For this purpose 0.5 milligram of indol may be employed and its compound dissolved with 10 or 15 c.c. of chloroform. The chloroform solutions to be compared are then placed in the cups

belonging to the Duboscq instrument. By means of this instrument it is possible to obtain fairly close correspondences between solutions of the condensation product prepared from equal quantities of indol. We have thus a standard for comparison with the condensation product obtained from the indol distilled from the fæces or other putrefactive material. It is believed that this method will prove more accurate than any now in use for the quantitative determination of indol and it can be recommended for simplicity.

The reagent with which the condensation with indol is brought about is at present difficult to obtain in this country, but it is our intention to request the leading dealer in chemicals in New York to import it and keep it in stock. Dr. T. Schuchardt of Görlitz, Germany, has recently listed the ammonium salt of the naphthaquinone compound, which one would expect to answer the requirements as well as the sodium salt.

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ON A RELATION BETWEEN SKATOL AND THE DIMETHYLAMIDOBENZALDEHYDE (PARA) REACTION OF THE URINE.

BY

C. A. HERTER.

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ON A RELATION BETWEEN SKATOL AND THE DIMETHYL-AMIDOBENZALDEHYDE (PARA) REACTION OF THE URINE.

BY C. A. HERTER.

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In 1901 Ehrlich [1] showed that most urines have a constitution which is capable of entering into color reactions with p-dimethylamidobenzaldehyde (Ehrlich's aldehyde). The reagent is employed in acid solution, generally with the aid of heat, and the cherry-red produced fades to orange-yellow on standing (rapidly in the presence of excess of acid), and is partly soluble in chloroform and completely so in epichlorhydrin. The appearance of the color is prevented by treating the urine with an aliphatic aldehyde like formalin, probably because the reacting substance in the urine is firmly united to the aldehyde and thus prevented from combining with the color-producing, aromatic aldehyde. It was noticed by Ehrlich that the intensity of the reaction is apt to be great in pathological urines, including those from patients with phthisis, typhoid fever, and chronic enteritis, and Clemens observed that persons with digestive disturbances are among those whose urines are likely to give a strong reaction. It has not been possible, however, for those who have heretofore worked on the subject, either to attach to the Ehrlich aldehyde reaction any definite clinical significance or to discover the chemical nature of the substance in the urine on which it depends. Still it appears that observers are agreed that in its intense form the reaction is pathological. Hence any light on the nature of the reaction is of medical as well as of biological interest.

While engaged in testing the urines of various persons with the Ehrlich aldehyde, it appeared to me that the urines of persons with urinary evidences (excess of indican and phenol) of exaggerated intestinal putrefaction are especially liable to exhibit

[1] *Medicinische Woche.*, 1901, No. 15.

the deepest cherry-color tints on being treated with the reagent, and that the urines of normal children (whose intestinal putrefactive processes are apt to be mild) often gave negative or almost negative results. This observation suggested the desirability of determining whether the intensity of the reaction is influenced by the presence of derivatives of putrefactive substances in the urine. Before making experiments in this direction, it was decided to determine whether the introduction of large amounts of free hæmoglobin simultaneously into the blood and into the intestine is followed by the intensification of the Ehrlich aldehyde reaction of the urine, due to the formation of a biliary chromogen (urobilinogen) and perhaps hæmopyrrol.[1] A dog of medium size was bled 150 c.c. and the defibrinated blood laked with distilled water. One-half the laked blood was infused intravenously and the other half was put in the stomach of the dog. The urine remained negative so far as the Ehrlich aldehyde reaction was concerned.

Experiments were next made with indol and skatol. Indol is well known to give a pink condensation product with Ehrlich's aldehyde. Skatol gives a violet or blue condensation product with the substance. The administration of 0.1 gram of indol to a dog was not followed by any alteration in the Ehrlich aldehyde reaction of the urine.

Experiments with skatol (Kahlbaum's) showed that the administration of the substance by the gastro-enteric path or subcutaneously regularly exerts a definite influence on the Ehrlich aldehyde reaction of the urine, as the following notes indicate.

Experiment 1.—Normal dog, weight about 18 lbs. Urine negative to Ehrlich's aldehyde. No reaction for skatol with concentrated hydrochloric acid. Bladder emptied. Gave 0.1 gm. skatol by stomach. Urine collected 1 hour later by catheter gives fairly strong cherry-red color with Ehrlich's aldehyde. The color goes over readily into chloroform. Urine collected by catheter three hours after skatol gives a moderate red with Ehrlich's aldehyde, which shakes out in chloroform. With concentrated

[1] This experiment was suggested by the views of Neubaur on the relation of hæmopyrrol (methylpropylpyrrol) to Ehrlich's reaction. See O. Neubaur, *Sitzungsbericht der Gesellschaft f. Morphologie u. Physiologie*, 1903, 2, p. 32.

hydrochloric acid the urine gives a strong skatol red reaction, but requires heat to bring it out. Urine collected by catheter five hours after skatol gives with Ehrlich's aldehyde a pink red, quickly becoming dirty brown, which shakes out moderately strong yellow red in chloroform.

Experiment 2.—Rhesus monkey (No. 1), weight about 5 lbs. Normal urine collected from cage and filtered. Sp. gr., 1.017. With Ehrlich's aldehyde gives slight reaction with light pink color. No skatol red reaction with cold concentrated hydrochloric acid. Received 0.1 gm. skatol subcutaneously. Urine of following seventeen hours collected from cage and filtered. Sp. gr., 1.025. Gives intense cherry-red reaction with Ehrlich's aldehyde. After dilution to sp. gr. 1.014 still gives a marked cherry-red reaction. On standing, this urine gives a strong skatol red reaction with cold concentrated hydrochloric acid.

Subsequent urines showed a falling off in the intensity of the aldehyde reaction. A urine collected from cage about forty-eight hours after the skatol injection, diluted to sp. gr. 1.016, gave a moderately strong Ehrlich reaction. Concentrated hot hydrochloric acid gave a slight skatol red reaction. A second injection of 0.1 gm. skatol was followed after eight hours by collection of a urine which, after dilution to sp. gr. 1.015, gave an intense cherry-red with Ehrlich's aldehyde. On cooling suddenly there occurred a brownish precipitate. Chloroform takes out a red coloring matter, leaving behind a purplish material. Formic aldehyde quite prevents the reaction. Concentrated hydrochloric acid gives a strong skatol red.

Urine collected twenty-four hours after the second injection still gives a strong aldehyde reaction. Sp. gr., 1.013.

Urine collected forty-eight hours after the second injection gives a slight Ehrlich reaction. Sp. gr., 1.015.

Urine collected four hours after a third injection of 0.1 gm. skatol gives an intense cherry-red Ehrlich's reaction. Sp. gr. not taken.

Urine collected eight hours after the third injection gives an extremely intense ruby-red color with Ehrlich's reagent.

Experiment 3.—Rhesus monkey (No. 2). Urine collected from cage, sp. gr., 1.020, diluted to 1.013, gives slight reaction with Ehrlich's aldehyde. Thirty hours after subcutaneous injection of 0.1 gm. skatol, urine diluted to 1.013 sp. gr. gives strong to intense Ehrlich's aldehyde reaction. Urine collected forty-eight hours after injection of skatol, diluted to sp. gr. 1.018, gives intense Ehrlich reaction. Urine collected seventy-two hours after injection (sp. gr., 1.011) gives moderate Ehrlich reaction. Urine collected ninety-six hours after injection (sp. gr., 1.017) gives moderately strong Ehrlich reaction and slight skatol red with concentrated hydrochloric acid (with slight heat).

Urine collected five hours after second injection of 0.1 gm. skatol subcutaneously (sp. gr., 1.012) gives intense cherry-red reaction with Ehrlich's aldehyde. The color goes over completely into epichlorhydrin. The reaction is checked by formic aldehyde. The urine gives an intense red with concentrated hydrochloric acid (with slight heat).

Experiment 4.—On Nov. 6, at 10 A.M., a healthy man passed urine (sp. gr., 1.020) giving a marked reaction with Ehrlich's aldehyde. He then took 0.025 gm. skatol (in solution) by the mouth, and at 1 P.M. this dose was repeated. At 1 P.M. urine was passed and diluted to sp. gr. 1.020. Ehrlich's reaction was of about the same intensity as in the preceding urine. The skatol red reaction was stronger. Urine passed at 3.15 P.M. (sp. gr., 1.026) was diluted to sp. gr. 1.023 and gave an intense Ehrlich reaction (distinctly more intense than in previous urines). Urines subsequently passed gave a strong or moderate Ehrlich reaction. During the morning of Nov. 8, the subject took 0.050 gm. skatol. Urines passed about two hours and about four hours after the last dose of skatol gave intense Ehrlich reactions. Urines passed subsequently gave weaker reactions, but on the following day a urine was obtained which gave an intense reaction.

Experiment 5.—Urine from a normal man collected at 8 A.M., Nov. 19, gave a slight reaction with Ehrlich's aldehyde—almost negative. Urine collected at 10.45 A.M. also gave a slight reaction. At 10.40 A.M. the subject took 0.05 gm. skatol in gelatin capsule with water. No symptoms were noted.

Urine secreted from 12 M.–4 P.M. gives marked reaction.
" " " 4 P.M.–6 P.M. " " "
" " " 6 P.M.–8 P.M. " " "

All the urines used in the above tests were diluted with water to sp. gr. 1.010. The increase in the intensity of the reaction after taking skatol was unquestionable.

The experiments here described show clearly that the administration of skatol was followed regularly by an intensification of the Ehrlich aldehyde reaction of the urine, and this result was especially striking in the case of Experiment 2 and Experiment 3. The intensified reaction did not differ from the spontaneous reaction in respect to the partial solubility of the cherry-red color in chloroform and the complete solubility in epichlorhydrin. It also resembled the spontaneous reaction in being abolished by the action of formic aldehyde. Whether the intensified color following upon the administration of skatol has the same spectroscopic characters as the spontaneous color reaction I am unable to say. There was in all the experiments a rough correspondence between the Ehrlich reaction and the reaction with concentrated hydrochloric acid, known as the skatol red or urorosein reaction, but no close parallelism between the former and the reaction of indican.

The urine of the subject of Experiment 4 habitually gives a

pronounced Ehrlich aldehyde reaction, and on this account the intensification after skatol is perhaps less convincing than in the case of the other observations. Nevertheless I consider the increase in this case also to have been distinct. The urine from the subject of Experiment 4 showed a distinct increase in the intensity of the reaction after the administration of skatol by mouth, but the increase was less than that noted in the experiments on monkeys.

These observations are recorded in order to draw attention to the fact that the appearance of a skatol derivative in the urine is capable of intensifying the Ehrlich aldehyde reaction of the urine or of forming the chief basis of a reaction indistinguishable from it by ordinary tests. I do not venture from the few experiments I have made to assume that the skatol derivative is the usual cause of the very commonly observed reaction with paradimethylamidobenzaldehyde, but it is worth while to investigate the reaction with this possibility in mind. It is not possible at present to feel certain that the intensification I have noted has all the characteristics of the true Ehrlich reaction. But the observed facts are of sufficient interest to make it desirable to study carefully the occurrence of skatol[1] in the intestinal con-

[1] The separation of skatol from indol is rendered comparatively easy by means of the following procedure. The intestinal contents or feces are distilled alkaline. The distillate is then distilled acid, and to this acid distillate is added a solution of β-naphthaquinone-sodium-monosulphonate. On the addition of potassium hydroxide in excess there occurs the formation of the blue condensation product of indol and the naphthaquinone compound, in the event of indol being present. On the addition of acid the color of the indol derivative is altered to yellow, and the solution is now distilled. The distillate contains skatol but no indol, provided skatol was present in the original distillate. The presence of skatol is readily ascertained by use of the paradimethylamidobenzaldehyde reaction, which gives a blue or violet color which can be shaken out by means of chloroform. Further details of this procedure will be given in a later communication, as it is believed that it will much facilitate the separation of skatol and indol from putrefactive material.

In a previous paper (*Jour. Exper. Med.*, March, 1905) it was erroneously stated that the naphthaquinone-sodium-monosulphonate gives a violet condensation product with skatol. This error is due to my having confounded α-methyl-indol with skatol, and the statements contained in the paper just mentioned are true of α-methyl-indol, but not of skatol. The fact that skatol does *not* give a condensation product with the

tents in relation to the behavior of the urine with Ehrlich's aldehyde.[1]

The tests of the urine were made for the most part with the aid of heat, although pronounced differences were also observed when the reaction was carried out in the cold.

Note during proof-reading.—Since the above was written, I have found that in persons whose urines give a strong Ehrlich reaction the feces contain skatol, but that in persons whose urines are negative to the aldehyde there can be recovered little or no skatol from the feces. No exception to this correspondence has yet been observed, and I am disposed to think that the absorption of skatol from the intestine is a common cause of the aldehyde reaction. The behavior of the urines to concentrated hydrochloric acid also supports this view.

I have further observed that the subcutaneous injection of tryptophane in monkeys causes an increase in the Ehrlich aldehyde reaction of the urine. In one instance the indican reaction was also increased.

naphthaquinone compound forms the basis of the method of separation, the outlines of which are given above.

[1] A good discussion of Ehrlich's dimethylamidobenzaldehyde reaction in the urine by C. E. Simon may be found in the American *Journal of the Medical Sciences*, cxxvi, p. 471, 1903. Bauer (*Zentralblatt für Innere Medicin*, 1905, No. 34, p. 833) brings forward evidence to support the view that the Ehrlich reaction depends on urobilogen. This view appears to me not irreconcilable with the experimental results obtained by me.

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CHARACTER OF THE BACTERIAL FLORA OF CARNIVOR AND OF HERBIVOROUS ANIMALS.

IN the course of the study of anaerobes of the human intestine it appeared desirable to learn something about the characters of the bacterial flora inhabiting the large intestine of various domestic and wild animals. It was noticed that in the dog, which is frequently exclusively carnivorous, the intestinal contents often showed the presence of large numbers of spores, spore-bearing bacilli and vegetative forms of anaerobes. The numbers present in the feces were noted to be especially large in some animals which had been exclusively fed on meat. A study of a grown cat fed upon raw meat showed the presence of Gram-positive vegetative anaerobes from one end of the digestive tract to the other. Flora derived from the stomach, small intestine and large intestine were inoculated and grown in bouillon flasks and showed an abundant production of methyl mercaptan as well as hydrogen sulphide. The numbers of colon bacilli present in this case were relatively small as compared with the anaerobes. The study of the colonies obtained on anaerobic plates showed that a large portion of the organisms present in the intestinal tract were *B. aerogenes capsulatus*. Intravenous infusion of these organisms into a rabbit which was afterwards killed and incubated showed in a high degree the typical gas-formation.[1]

Observations on other cats showed the ence of considerable numbers of spore-h bacilli and free spores, sometimes in c in addition to vegetative forms of ana The position of these spores and spore-h bacilli has not been established in a ba logical sense. Observations were also upon the intestinal contents of the wolf and lion. Several different tigers were s and the observations were not confined examination of one lion and one wolf. material from the lion showed the prese many free spores. It also showed the pr of considerable numbers of Gram-p bacilli, suggesting *B. aerogenes caps* Gram-stained preparations from wolves s findings similar to those observed in th except that the spore-holding bacilli were numerous. The findings in the case o posedly healthy tigers were not esse different from those in the case of th and lion. In the case of one tiger, su from osteomalacia, greatly impaired nu and loss of strength, the microscopical derived from several different samples o revealed the presence of immense numb free spores and smaller numbers of imm Gram-negative spore-holding bacilli. spores developed into organisms which sessed all the generally known cultura biochemical characters of *B. aerogene sulatus*, including the ability to dev high grade of gas-formation in rabbi jected and incubated.

It was found that bouillon cultures mixed fecal flora from the lion, tiger and cat all developed quickly a sufficient

[1] The incubation method of Welch and Nuttall is based on their observation that the gas bacillus produces gas abundantly in the blood, organs and tissues of rabbits killed a few minutes after intravenous injection. Here the blood and tissues of the rabbit act as a peculiarly favorable culture medium for the growth of the gas bacillus, the latter having been thoroughly spread by the blood through the body, and the conditions being anaerobic. A suspension of the feces to be tested is prepared by grinding 1 gram of the fresh material with 9 c.c. of 0.85 per cent. salt solution and filtering through absorbent cotton. One c cubic centimeters of this suspension are th jected intravenously into a rabbit which is killed and incubated for five hours at 30° C

ity of methyl mercaptan to give promptly a ery well developed reaction with isatin-sulhuric-acid.

Experiments were made with the mixed ecal flora from these carnivorous animals to etermine their pathogenicity when injected nto the subcutaneous connective tissue. It ould have been better to have worked with ure cultures of the anaerobes in question, ut opportunity has not yet arisen to isolate hem. The result of the inoculations into uinea-pigs was the same in each instance. 'he animals died within twenty-four hours nd usually in fifteen to eighteen hours. At utopsy the subcutaneous connective tissues ere hemorrhagic, œdematous and showed ecrotic changes which extended in some intances to the muscles. Gas-formation was ot usually noted as a prominent feature. 'hese pathological alterations were not conned to the site of inoculation but had exnded to the subcutaneous connective tissues hroughout the body and were especially proounced in the axillæ and in the groin. It is nnecessary to enter here into the details as the character of the organisms recovered rom these lesions.

We may contrast with these findings the bservations made upon herbivorous animals, ncluding the buffalo, goat, horse, elephant nd camel. In the case of the camel, elephant nd horse the preponderant bacteria in the ram-stained fields were small Gram-negative rganisms which were regarded as special orms of *B. coli*. In the case of the goat the elds contained some Gram-positive bacteria nd of the Gram-negative ones a considerable umber were of considerably greater length han the dominant small forms which were egarded as belonging in the class of colon acilli. In the case of the buffalo, mixed elds were found as regards the Gram-staining nd many of the positive organisms were ound to be small diplococci and small bacilli. n none of these animals were seen any organsms suggesting *B. aerogenes capsulatus* exepting in the case of the buffalo where the *umber of bacilli* of this type was very small. *re-holding organisms were not observed, moderate numbers of free spores* were noticed in all the fields except those from th elephant. In the fields showing the larges number of spores their occurrence was far les frequent than in the lion, tiger, wolf or cat

The mixed flora of these different herbivo rous animals, grown upon peptone bouillon failed to show the production of methyl mer captan excepting in the case of the horse where a moderate reaction was obtained.

Observations were also made upon the effec of suspensions of the mixed flora from her bivorous animals when injected subcutane ously. The quantities of suspension use were usually about twice as great as in th case of the suspensions from the carnivorou animals. With the exception of the suspen sions obtained from the horse, the pathogen icity of these suspensions was found to b slight, the guinea-pigs frequently living tw or three days or entirely recovering. In th horse were found hemorrhagic and œdematou lesions with necrosis, similar to those foun in the carnivorous animals. These lesion were, however, less pronounced than in th case of the suspensions from the carnivorou animals. In the case of the elephant a con siderable quantity of fibrinous exudate wa found about the point of inoculation. N œdema or necrotic change was observed in th subcutaneous tissues.

A further confirmation of the radical differ ences existing in the intestinal tracts of car nivora and herbivora is furnished by a serie of observations with the Welch-Nuttall incu bation test. Suspensions were made from th feces of all the types of animals mentione and equal quantities of these suspensions wer infused intravenously into a series of living rabbits. The rabbits were then quickly kille and incubated. On examination after twenty four hours it was found that all the rabbit infused with suspensions from carnivor showed in an extreme degree the characteristi putrefactive changes in the liver, cellular tis sues, etc., induced by pure cultures of *B aerogenes capsulatus* or of the bacillus o symptomatic anthrax. The rabbits infuse with suspensions made from the feces of th herbivor[illegible] sim[illegible] very muc slighter [illegible] he result

for each group of animals separated the herbivora sharply from the carnivora. Examination of the livers showed the number of bacteria in the carnivorous series to be many times greater than in the herbivorous series. The microorganisms were regarded as being almost certainly *B. aerogenes capsulatus* on account of their morphology and failure to sporulate. The bacilli of symptomatic anthrax readily sporulate in the incubated rabbits. The gas-bacillus (*B. aerogenes capsulatus*) does not sporulate under these circumstances.

These differences in the appearance and behavior of the bacteria derived from typical carnivora and herbivora suggest that the habit of living upon a diet consisting exclusively of raw meat entails differences in the types of bacteria that characterize the contents of the large intestine. The occurrence of considerable numbers of spore-bearing organisms in the carnivora points to the presence of anaerobic putrefactive forms in great numbers. The results of subcutaneous inoculations into guinea-pigs bear out this view and indicate that the numbers of organisms capable of producing a hemorrhagic œdema with tissue necrosis, with or without gas-production, are very considerable. Unfortunately, the data pertaining to the biological properties of these pathogenic anaerobes are at present insufficient to permit us to classify them or to say more of their nature than that they are organisms representative of a definite group of putrefactive anaerobes which make butyric acid and hydrogen and exert a peptonizing action upon living tissues. Nevertheless, the observations here recorded are of much interest in relation to the bacterial processes and nutrition of herbivorous[2] as distinguished from carnivorous animals and are signi[illegible] furthermore for the interpretation of [illegible] terial conditions found in man. The que[illegible] arises whether the abundant use of meat [illegible] a long period of time may not favor th[illegible] velopment of much larger numbers of s[illegible] bearing putrefactive anaerobes in the [illegible] tinal tract than would be the case were a [illegible] ferent type of proteid substituted for me[illegible]

[2] Many of the herbivora yielded mixed flora incapable of making gas on dextrose bouillon.

Inquiries made of Dr. Blair, the patho[illegible] at the New York Zoological Park, elicite[illegible] fact that while, upon the whole, the car[illegible] rous animals are apt to live somewhat l[illegible] than the herbivorous animals of about [illegible] size, the carnivora are much more like[illegible] develop conditions of advanced anæmia i[illegible] later years of their lives than is the case [illegible] the herbivora. Dr. Blair states that it is [illegible] in the later years of life for the carnivo[illegible] show a much diminished volume of bloo[illegible] at least a moderate fall in the hemog[illegible] Instances are stated to be not uncomm[illegible] which a pernicious type of anæmia has [illegible] oped in the carnivora. On the con[illegible] among the herbivora it is said that prono[illegible] anæmias are very occasional. The exa[illegible] of severe anæmia encountered among the [illegible] bivora were said by Dr. Blair to be in [illegible] all instances referable to gross animal [illegible] sites.

The information now available ind[illegible] that man occupies a position between the [illegible] bivora and carnivora with respect to the [illegible] bers of putrefactive anaerobes that are p[illegible] in the digestive tract and their proporti[illegible] the total number of bacteria. The infl[illegible] of a purely vegetable diet on the one [illegible] and of a strict meat diet on the other, [illegible] these anaerobes, is much in need of ca[illegible] investigation.

C. A. Her[illegible]

ON GAS PRODUCTION BY FECAL BACTERIA GROWN ON SUGAR BOUILLON.

BY

C. A. HERTER AND HERBERT C. WARD.

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ON GAS PRODUCTION BY FECAL BACTERIA GROWN ON SUGAR BOUILLON.

By C. A. HERTER and HERBERT C. WARD.

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The ingenious and helpful studies by Professor Theobald Smith of pure cultures of bacteria grown in fermentation tubes suggested to us that something might be learned of the physiological attributes of the mixed bacteria of the human feces when these are grown in health or disease in the anaërobic portion of fermentation tubes. We desire to record here briefly the results of observations on the gas production by mixed fecal bacteria introduced (in a water-suspension designed to secure representative bacteria) into fermentation tubes containing sugar-peptone solutions. The observations on gas production were recorded at the end of about forty-eight hours' sojourn in the incubator at 37° C., and are given in terms of the height of the columns of gas in millimeters. Four sugar-peptone media were used.

The basis of the media was a bouillon medium to which were added different sugars in a concentration sufficient to give in each instance a concentration of two per cent. The sugars used were dextrose, a levulose-dextrose mixture (Schering's diabetin) lactose, and saccharose. Nearly uniform results in gas production were obtained in duplicate series when care was taken to insure a uniform distribution of the inoculated fecal bacteria.

The gas production by the mixed, unisolated fecal bacteria of normal individuals was found to be influenced somewhat by dietetic conditions and age. Presumably healthy, breast-fed children showed a smaller gas production than children who were bottle-fed or than children or adults on mixed diet. In the present communication reference will be made mainly to the gas production by the fecal bacteria of normal and pathological adults and of children who are no longer infants.

The average gas production noted in sixteen observations on the fecal bacteria of normal individuals was 103.65 mm. for the

four tubes. The largest gas production among these was 138, 137, and 129 mm., the smallest, 65, 69, and 72 mm.[1] As a rule, the largest gas production was in the lactose-peptone tube, the smallest, in the saccharose tube. The averages of gas production for the different individual tubes are as follows:

Dextrose	Levulose-dextrose	Lactose	Saccharose
26.75 mm.	27.5 mm.	29.9 mm.	19.5 mm.

The gas formed under the conditions that have been described was in some instances subjected to the absorption test by a caustic potash solution. The proportion of gas absorbed varied somewhat in the different sugar-peptone tubes. It may be said that, as a rule, from about one-quarter to one-tenth of the total gas (representing carbon dioxide) was absorbed. The proportion of carbon dioxide production was about the same in the cases where gas formation was inhibited.

Some observations have been made on the gas production by pure cultures of bacteria normally inhabiting the gastro-enteric tract. For example, one strain of B. coli communis gave 90 mm. of gas in the four tubes, while another strain gave 92 mm. B. lactis aërogenes gave 76 mm. All these values are somewhat below those obtained by growing the mixed fecal bacteria from presumably normal persons. Greater gas production was noted where B. coli was grown with B. aërogenes capsulatus.

The fact which we desire to bring forward in this communication is that there are pathological conditions in which the production of gas by the mixed fecal bacteria is distinctly less than is usual for normal persons. The feces from a number of persons showing the evidences of excessive intestinal putrefaction (putrefactive products in the feces or derivatives of putrefactive products in the urine) have been studied with respect to their gas production. It was found that a somewhat diminished gas production is a not infrequent accompaniment of various digestive disorders. A pronounced reduction in gas formation is less common, and when persistent is apparently associated with graver clinical manifestations.

Thus in a man of thirty-two years, suffering from pernicious anæmia, the gas production in the four tubes was only 30 mm.

[1] The anaërobic portion of the tubes used by us is about 9.5 cm. in length.

This was soon after entering the hospital. About a week later, during a diarrhœal period the gas production rose to 100 mm. but declined again later to 60 mm.

In another patient with pernicious anæmia, the feces from a diarrhœal stool gave 155 mm. of gas; some weeks later a formed stool gave bacteria which produced 45 mm. of gas. In a third patient with the same disease, a diarrhœal stool gave 95 mm. of gas, but later the fecal bacteria from a formed stool gave 70 mm. only.

In another patient with pernicious anæmia, the semi-solid stool contained bacteria which gave 80 mm. of gas. As the patient improved, under the influence of rest and care in diet, the gas production increased to 90 mm. and later, coincidentally with further improvement, to 112 mm. Another patient with pernicious anæmia, a child of one year, gave on one occasion 35 mm. of gas, on another 38 mm.

Very low gas production was repeatedly observed in a severe case of diabetes on the verge of coma.

The smallest gas production has been noted in bottle-fed children suffering from marasmus.[1] In several instances the fecal bacteria from such children have failed to make a volume of gas measurable by ordinary methods. In one highly anæmic child in a marantic state the gas production amounted to 18 mm. when the first observation was made. After a week in bed, on a carefully chosen diet, the gas production was 105 mm. One week later it was 120 mm., and two weeks later 96 mm. The increase in gas production coincided with a striking improvement in nutrition and in the blood picture.

A marked fall in gas production has been noted during fever in a case where previous to the rise in temperature the gas production was large.

The explanation of the phenomena recorded here is not yet clear, but it appears likely that it is attributable at least in some instances to an interference with the normal gas-producing properties of organisms of the B. coli communis group. One may think of the gas-forming kinds of bacteria as actually dying out to a large extent in the lower bowel, or one may imagine them

[1] Our observations on bottle-fed children are not sufficiently numerous to enable us confidently to give the normal gas production for them. It appears to vary from 50 to 100 mm.

to be simply inhibited in their growth by the presence of bacteria which are limited gas-producers. The view that the gas-makers of the upper bowel are replaced in the lower bowel very largely by organisms with other physiological character is substantiated in a noteworthy manner by the results of studies with the gram stain. Bacteria of the B. coli group are gram negative, and where we have preponderantly gram-negative stools containing, B. coli in large numbers one would expect an average gas production by fecal bacteria grown on sugar bouillon. This has been actually the case. On the other hand, gram-positive feces containing bacteria of the B. coli type are small gas-producers. A special instance of a physiological flora which is able to make little gas and is gram-positive is that of the breast-fed infant in which B. coli has not become established and in which B. bifidus (Tissier) and B. acidophilis (Moro) are dominant. In adults and in children on cow's milk a gram-positive stool is usually not physiological, and in such cases it may be that small gas production is partly dependent on the inhibiting action of some pseudo-parasitic organism or combination of organisms.

We have repeatedly observed a change in the fecal flora from a gram-positive to a gram-negative character and with this change an increase in gas production. In an autopsy on a child dead of pneumonia, the following conditions were observed with respect to gas production. Bacteria from the stomach gave 40 mm. of gas; bacteria from the duodenum, 94 mm. of gas; cultures from the jejunum gave 78 mm. of gas, and the same amount was obtained from the cultures from the ileum; and finally bacteria from the rectum gave only 22 mm. of gas.[1] In the rectum the bacteria were gram-positive; above the rectum, mainly gram-negative.

We have not yet been able to reproduce experimentally the impaired gas formation by combining with the colon bacillus some organism capable of inhibiting its gas-producing qualities. A definite association between gas production by the fecal bacteria under anaërobic conditions of growth and special conditions of intestinal putrefaction has not been established.

[1] This observation harmonizes with the fact that the diarrhœal flora of the human intestinal contents generally give more gas than the bacteria derived from formed movements from the same individual.

We believe that further studies of the phenomena here described will prove to be of biological interest and of value in clinical investigations of intestinal disorders.

Note.—A great increase in gas production was noticed in a monkey which developed diarrhœa after feeding with cabbage. This observation is in accord with what we have noticed in connection with human diarrhœa.

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ON BACTERIAL PROCESSES IN THE INTESTINAL TRACT IN SOME CASES OF ADVANCED ANÆMIA, WITH ESPECIAL REFERENCE TO INFECTION WITH B. AEROGENES CAPSULATUS (B. WELCHII).

By C. A. HERTER.

(Received for publication May 10, 1906.)

I have thought it worth while to bring together here a number of observations relating to bacterial processes in the intestinal tracts of persons suffering from severe forms of anæmia, in the belief that they afford a clue to the etiology of many cases of advanced blood disease that are ordinarily described as "primary." Most of the instances which form the basis of this study have given the blood-picture of so-called idiopathic, pernicious anæmia.

The observations relate especially to seventeen cases of anæmia. Of these nine showed those changes in the morphology of the red cells (including the presence of megaloblastic forms) which are regarded as most characteristic of primary pernicious anæmia (Cases I, III, V, VII, VIII, X, XII, XIII, XVII). In four other cases (Cases II, IV, VI, and IX) the diagnosis of pernicious anæmia was made but appears less certain as megaloblasts were not seen. Of the remaining four cases one is an adult from whose history and blood-picture a distinguished consultant leaned to the diagnosis of pernicious anæmia, while another practitioner, upon the same evidence, suspected that he was dealing with an anæmia secondary to gastric carcinoma (XV). Another case is one of severe anæmia which was regarded as secondary, but for which no explanation could be found at autopsy (Case XI). Another case is that of a young child in the care of Dr. Holt, whose history is especially instructive as showing the rapidity of the process of blood regeneration upon the subsidence of a subacute intestinal process to which the anæmia was apparently secondary (Case XVI). The remaining case (XIV) occurred in a child of three

years of age and is probably a splenic anæmia, secondary to some unknown infection. I feel especially indebted to Drs. Spalding, James, Thatcher, and Loomis for placing at my disposal the opportunities for studying the greater number of the patients and for furnishing me with the clinical notes. Dr. Fred. Shattuck of Boston has placed me under obligations for his reports on Case XII.

It is a fact well recognized by those who have studied considerable numbers of patients with pernicious anæmia, that these persons are liable to pronounced derangements of digestion, such as diarrhœa. Among the cases collected here, diarrhœa was a frequent occurrence. It was noted also that even where diarrhœa was absent, the feces were not well formed but were usually of a semi-solid, tarry consistence. And it is worthy of mention that among the many specimens which were sent to the laboratory from persons with pernicious anæmia, since the beginning of the present study, there were very few instances in which the stools have been well formed and of firm consistence throughout or in large part.

Routine microscopical examinations of the stools were made by Dr. W. R. Williams with reference to the presence of meat fibres, vegetable residue, starch, phosphates, free fat, fatty acids, and soaps. Unfortunately the examinations were not made with a sufficiently full knowledge of the nature of the diet in each case to make it safe to form a generalization with reference to the features mentioned. It was noticed that meat fibres and vegetable residue were very abundant in some instances, but the significance of these facts is uncertain, partly because the dietetic conditions are not known in detail, partly because the habits of the patients with respect to mastication were not recorded. An excessive amount of mucus was noted in Cases X, XI, and XII. In Case XII its presence was a regular feature.

Occurrence of Phenol, Indol, and Skatol in the Feces and of their Derivatives in the Urine.

In the present study considerable attention was given to the amounts of the aromatic cleavage products, indol, skatol,

and phenol in the feces and to their derivatives in the urine. These bodies, as is well known, are common accompaniments of putrefactive decomposition in the intestine, and it seemed desirable to learn whether in severe anæmic states, not secondary to obvious pathological processes, there is anything noteworthy as to their production.

It is possible to dismiss in a few words the observations that relate to phenol, or, more correctly, to phenolic substances—phenol and paracresol not being distinguished from each other by the method of determination employed.[1] In the following list of cases the phenol values are expressed in milligrams per hundred grams of feces.

No. of Case	Phenol in Mgms. per 100 Gms. Feces
I	2.2; 3.6.
II	14.4; 15.1; 12.8.
V	7.7.
VI	6.6; 6.8.
VII	12.9.
VIII	5.2; 13.1.
X	13.2; 4.1.
XII	10.; 5.7; 12.7; 9.0; 19.5; 11.4; 4.9; 2.2.
XIII	5.7.
XIV	7.8; 5.9.
XV	9.4; 4.5; 11.2
XVII	2 5.

The phenol content of normal feces varies within wide limits and even in the case of presumably normal children and adolescents, with no indications of digestive disorder, may amount to 12 milligrams in 100 grams of fresh material. It is difficult to fix the normal limits for phenol but it is clear that in our cases of anæmia there are none in which the phenol content of the feces was strikingly or persistently above the normal. In Case XII, 19.5 milligrams were found on one occasion but this was evidently an exceptionally high value. The largest quantities we have found were observed in the case of a child of eight years who was slowly convalescing from chronic intestinal indigestion of

[1] Kossel and Penny.

such severity as to retard growth and in whom a moderate degree of anæmia (hæmoglobin, 65 per cent.) had developed. Other children suffering from similar clinical manifestations have given uncommonly high results.[1]

In a number of instances observations were made on the phenol of the urine, but there are only a small number of quantitative determinations. In a large number of cases the distillate from the urine was tested with Millon's reagent, the color reaction being described as slight, moderate, or strong. Many times the urine was tested directly in the cold with Millon's reagent. If the urine contains considerable phenol potassium sulphate, this salt is hydrolyzed under these circumstances and the liberated phenol reacts in a characteristic way.

In Case I the phenol reached 85 milligrams on one occasion, 61 on another, and 27.5 on a third, the results being calculated for twenty-four hours; in Case II it was very strong, on one occasion reaching 82.9; in Case XII it was strong on two occasions (on one of these 56.42 milligrams in twenty-four hours or 36.4 milligrams in 500 c.c. of urine) but was subsequently moderate or slight on three occasions. In Case X eight examinations were made. On three occasions the distillate was negative, twice it gave a slight reaction, once a moderate reaction, and twice a strong one. In Case XIV (in which the diagnosis of pernicious anæmia was extremely doubtful) there were two negative examinations. In Case XV the reaction was very strong, reaching 96 milligrams in twenty-four hours on one occasion and 130 milligrams on another.

Summarizing the observations with respect to phenol it may be said that the phenol values for the feces have in general not been remarkably high, but that the phenol output in the urine has in a majority of instances been greater than normal. The urine in most of the cases exhibited a strong phenol reaction (Cases I, II, IV, VII, and IX) but in Cases X and XII the phenol excretion cannot be said to have ruled high, although these were well defined examples of pernicious anæmia with evidence of

[1]A special report will be made on the bacteriological and chemical conditions in cases of this type.

persistent disorders of intestinal digestion. In two of the five cases in which the phenol reaction from the urine was strong it was exceptionally intense and in all of these cases was sufficiently pronounced to point to excessive intestinal putrefaction. Comparing the phenol reaction in these cases with those obtained from normal children and young adults living under hygienic conditions, the reactions must be regarded as excessive, but they were less marked than in many cases of chronic intestinal indigestion in children and adults, excepting Cases I, II, and XV, in which the values were very high.

The observations relating to the indol of the feces and to the indican of the urine are more numerous than those having to do with phenol. They are also of greater significance for the reason that while the urine is never free from phenolic derivatives, even in health, it is often quite free from indican. Indeed an examination of many urines from children and adolescents in good health, and on a hygienic, mixed diet, makes me believe that anything more than a trace of indican in the urine indicates a departure from ideal conditions of intestinal digestion. The formation of putrefactive products in the intestine and their excretion by the urine is in such small quantities, in the cases to which reference is made, that one cannot avoid the conviction that normal digestion is associated with only slight putrefactive decomposition, despite the sojourn of very large numbers of living bacteria in the large intestine.

The indol content of the feces was measured by means of the naphthaquinone-sodium-monosulphonate method[1] and is in some instances expressed in milligrams. The indican of the urine was not quantitatively determined but was roughly gauged by the Obermeyer reaction. A point to which some attention was given is the relation between the intensity of the indican reaction and the quantity of indol recovered from the feces. No definite proportionality between these was noted; indeed, in a number of instances results were obtained which appeared to negative any close relationship between them.

The results obtained are recorded in the following table:

[1]Herter and Foster, "A Method for the Quantitative Determination of Indol," This Journal, i, p. 257, 1906.

No. of Case	Date	Quantity of Indol in 100 Grams of Feces	Indican Reaction of Urine	Further Data
I	Mar. 23, '06 Mar. 30, '06	Trace "	Very strong Negative	Feces unformed
II	Mar. 21, '06 Mar. 28, '06	Negative "	Strong Trace	Feces formed but soft
III	Mar. 21,'06 Apr. 30, '06	Moderate [0.64 mg.] Negative	Negative Strong; deep purple	Feces soft, containing lumps.
IV	Dec. 18,'05 Jan. 5, '06	Strong Negative	Moderate Negative	Diarrhœal movement
VI	Mar. 17, '06 Mar. 26, '06	3 mg. 3.09 mg.	Strong Negative	Formed and liquid feces
VII	Jan. 18, '06 Mar. 15, '06	Trace - - - -	- - - - Moderate	
VIII	Dec. 30, '05 Jan. 9, '06 Apr. 16, '06	Trace Negative Faint trace	 Slight Negative	
IX	Dec. 4, '05	Very strong 13.0 mg.	Strong	
X	Nov. 28, '05 Nov. 30, '05 Dec. 5, '05 Dec. 8, '05 Dec. 12,'05 Dec. 18,'05 Dec. 28, '05 Feb. 1, '06 Mar. 14, '06 Mar. 27, '06	Very strong 46.9 mg. Negative 11.4 mg. 5.5 mg. Strong trace Negative Slight 0.27 mg. - - - -	Strong Strong Moderate Very strong Strong Negative Slight Strong	Period of diarrhœa.

No. of Case	Date	Quantity of Indol in 100 Grams of Feces	Indican Reaction of Urine	Further Data
XII	Dec. 25,'05	Strong, [7 mg.]	Strong	
	Jan. 2, '06	Trace	Strong	
	Jan. 15,'06	Negative	Strong	
	Feb. 1, '06	Slight	Very strong	
	Mar. 14, '06		Strong	
	Mar. 17, '06	Slight	Negative	
	Mar. 31, '06	Moderately strong		
	Apr. 5, '06	7.7 mg.	Faint trace	
	Apr. 27,'06	28.62 mg.	Slight.	
XIII	Apr. 17,'06	Moderate	Strong	
XIV	Jan. 3, '06	Trace		
	Jan. 10, '06	Faint trace	Negative	
XV	Mar. 30, '06	Negative	Strong; purple color	
	Apr. 4, '06	- - - -	Strong	
	Apr. 30,'06	Negative	Very strong	
XVII	May 5, '06	Faint trace	- - - -	

A consideration of the tabulated data shows that the condi tions were widely varying with respect to the fecal indol and the urinary indican, but that in a majority of the cases there was a distinctly excessive excretion of indican. It sometimes happened, as in Case X, that the indican temporarily disappeared from the urine during a period of diarrhœa and there is little doubt that in such cases the negative reaction was dependent on the rapid passage of the contents of the small intestine through the large intestine. Diarrhœas are frequent among persons with pernicious anæmia and may be responsible for some of the negative results as to indican.

It is noteworthy that in one instance (Case X) in which the feces contained a large quantity of indol and the urine gave a strong indican reaction, there was an abrupt falling off in these when lavage of the colon was begun. Coincidently with this there was a rapid improvement in the condition of the patient

and a strikingly rapid rise in the number of red blood cells and in the quantity of hæmoglobin.

In one case (XIV) the urine was free from indican although diarrhœa was absent. The feces contained very little indol.

A feature of some interest as an evidence of putrefaction is the frequent occurrence of skatol in feces of persons suffering from severe anæmia of "idiopathic" origin. Among normal persons on mixed diet, the presence of more than a trace of skatol in the feces appears to be an exceptional occurrence. In Case I skatol was fairly abundant (3 milligrams in 100 grams of feces) although only a trace of indol was present. In Case II skatol was present in traces on two occasions, although indol was absent. In Case III skatol was not found. In Case IV skatol was absent on one occasion when indol was abundant; at another time the skatol reaction was strong while indol was negative. In Case VI indol and skatol were both fairly abundant. In Case VII the skatol reaction was moderately strong but indol was detected only as a trace. The same is true of one sample from Case VIII but in a second specimen from this patient (ten days later) indol was unobtainable and skatol was abundantly present. In Case IX skatol was negative while indol was abundant. In Case X skatol was regularly absent (8 examinations) although indol was frequently present. In Case XII skatol was present only once in five times at which examinations were made. The largest quantity of skatol recovered from any pathological feces was in a case of diabetes on the verge of coma. Here only skatol was obtained, indol being wholly absent.[1] In Case XVII considerable skatol was obtained from the feces but no indol.

It is not yet clear why skatol makes its appearance in the intestinal contents in the course of putrefactive disturbance. Like indol it must be regarded as coming from the tryptophan yielded by proteid, but what the conditions are that determine the formation of one of these substances rather than the other we do not know. In ordinary putrefaction indol is usually

[1] The determination of skatol is made colorometrically by means of the reaction with paradimethylamidobenzaldehyde after distillation from a mixture from which nearly all of any indol present has been removed by precipitation with β-naphthaquinone-sodium-monosulphonate. The details of the method will be soon published.

an early product and skatol, if found at all, a much later one. From the putrefaction of peptone and bouillon media by various micro-organisms, individually and in various combinations, for short periods, I have been unable to obtain skatol. One medium from which I have obtained skatol by putrefaction is one prepared from sheep's brains. This origin of skatol from decomposing brain tissues was known to Nencki many years ago, but no explanation of it has been given. Skatol also appeared in a medium containing salts, tryptophan, alanin, and phenylalanin, after incubation with fecal bacteria. But by far the greatest concentration of skatol in a putrefactive culture was found after two weeks' growth of fecal bacteria in a peptone bouillon medium which had been enriched by the addition of a watery extract prepared from fresh asparagus. In one case the fecal flora from a normal pig were used. Here no indol was formed but a very large quantity of skatol. In another experiment in which the fecal bacteria were derived from a diabetic patient, there was very large production of skatol and slight production of indol.

A consideration of the skatol content of the feces carries one to the question of the significance of the paradimethylamidobenzaldehyde reaction of the urine. It is well known that on the addition of an acid solution of Ehrlich's aldehyde to certain urines a cherry-red reaction is obtained, sometimes in the cold, more often only on the application of heat. The cause of the reaction has been the occasion of some discussion. Erhlich[1] was inclined to attribute it to glycosamin; Neubauer[2] and Bauer[3] refer it to urobilinogen which has passed from the intestine into the urine. I have found that the administration of skatol to men and to monkeys is followed by some intensification of the aldehyde reaction and believe that where skatol is found in fair abundance in the intestine it may contribute to the reaction. It cannot, however, be claimed that in the group of anæmias which is under notice here, there was a close relation between the occurrence of skatol in the intestine and the capacity of the urine to give the aldehyde reaction. The correspondence has failed in either direction in some instances, that is, the intestine

[1] *Medicinische Woche*, 1901, No. 15.
[2] *Sitzungsber. d. Gesellsch. f. Morph. u. Physiol.*, 1903, 2, p. 32.
[3] *Zentralbl. f. inn. Med.*, 1905, No. 34, p. 833.

has sometimes contained considerable skatol while the urine has given no aldehyde reaction, and the reverse of these conditions has also been encountered. In about one-half of the urines from the anæmia group the Ehrlich aldehyde reaction has been obtainable, though not without the aid of heat. The strongest reactions I have observed have been obtained not in the case of the patients forming this group but in persons without marked anæmia, who were suffering from chronic disturbances of intestinal digestion. In the cases of anæmia now under consideration a marked aldehyde reaction of the urine has been less often obtained than a marked reaction for indican. It has also been evident that this aldehyde reaction bore no relation either to the indican reaction or to the phenol content of the urine.

The Dimethylamidobenzaldehyde Reaction of the Feces.

If one extracts human feces with an aqueous sodium chloride solution (2 grams of feces to 20 grams of 0.85 per cent. sodium chloride solution) the extract will usually yield a color reaction with a suitably prepared acid solution of Erhlich's aldehyde.[1] In the case of healthy children or adolescents on a mixed diet the color obtained is usually a light rose, and may be very faint. Between this light tint and a very deep cherry-red, all transitions are met.[2] It is noteworthy that the feces from the anæmia patients under consideration have regularly yielded very strong or intense Ehrlich aldehyde reactions, the only exceptions to this rule having been met with during periods of diarrhœa. It sometimes happens that a patient with chronic intestinal indigestion or a person on a strict proteid diet gives an intense Ehrlich aldehyde reaction. Normal adults on mixed diet give a moderate reaction and (in cases where the ethereal sulphates, phenol, and indican of the urine run low) often only a faint one.

The explanation of the chemical basis of the Ehrlich aldehyde reaction of the feces is not yet wholly satisfactory. Baumstark[3] thought it could be ascribed to the indol of the feces and based

[1] Water, 270 c.c., concentrated H_2SO_4, 30 c.c., Ehrlich's aldehyde, 15 grams.

[2] We have employed a graded color scale in order to record our results with some degree of accuracy.

[3] *Münch. med Wochenschr.*, No. 17, 1903; also, *Arch. f. Verdauungs-krankh.*, IX, 1903.

a quantitative method for indol on the reaction. Bauer,[1] however, showed that the feces contain another substance which reacts with the aldehyde and claims that this substance is urobilinogen. I reached a similar conclusion independently, after noticing that the feces in one instance gave an intense red reaction after the indol had been distilled off. In other cases a strong reaction was obtained in spite of the fact that the feces were free from indol from the outset. That the reaction from this non-volatile part of the feces depends wholly on urobilinogen does not appear to me to have been convincingly shown. It is true, however, that one may reduce urobilin (Schuchardt's) with alkali and zinc dust and thus obtain a substance which gives a stronger and more characteristic Erhlich aldehyde reaction than the urobilin itself. Probably both urobilinogen and a skatol derivative are implicated in the Ehrlich aldehyde reaction of the urine, but it is possible that other substances are also concerned.

Acids and Bases of the Feces.

Some attention has been paid to the quantity of volatile acids present in the feces of anæmic and other patients and also to the volatile bases present. The titration values have usually been such as to show that the ammonia (of which the bases mainly consist) almost exactly neutralizes the acetic, propionic, and butyric acids of which the acid in the distillate mainly consists. The values for volatile acids and bases in anæmias have not shown uniform deviations from normal values. Nevertheless it is common to find rather high values for the volatile fatty acids of the feces of persons with "primary" pernicious anæmia. Such high values are not confined to cases of this sort but are frequently observed in the intestinal contents of persons suffering from chronic intestinal indigestion, associated with an excessive excretion of phenyl-potassium sulphate and indoxyl-potassium sulphate by the urine. In such instances of chronic intestinal indigestion with excessive putrefaction there is almost invariably present some degree of anæmia. The hæmoglobin may not be greatly reduced. It is not exceptional to meet with persons who show the intestinal conditions just mentioned, but whose hæmoglobin is not below 70 or 80 per cent. A careful examina-

[1] *Loc. cit.*

tion in such instances will, I think, often reveal the fact that there is more real anæmia than is indicated by a consideration of the hæmoglobin alone, there being in such cases unequivocal signs of a diminished volume of blood. In both these milder anæmias and in the more severe forms, it is characteristic to find the various signs (to be described later) of the presence of an excessive degree of what may be designated the saccharo-butyric type of putrefaction—a process attended by the excessive formation of butyric acid (perhaps also propionic, valerianic, and caproic). I have been able to show that in many of these cases of excessive intestinal putrefaction attending milder or more severe types of anæmia, the higher volatile fatty acids are considerably increased in amount.

The following table includes some of the results obtained from a study of fatty acids of the feces.

TABLE SHOWING THE QUANTITY OF VOLATILE FATTY ACIDS IN 100 GRAMS OF DRIED FECES (IN TERMS OF OXALIC ACID).

Clinical Data	Volatile Fatty Acids—Grams	Other Data
I. Observation on normal child on mixed diet, showing minimal indications of putrefactive intestinal decomposition.	0.095	
II. Observation on patient aged 16 with continuous fever (influenza) of 39°C–40°C and temporary increase in intestinal putrefaction. Mixed diet.	0.426	
III. Observation on adult patient with primary pernicious anæmia (Case XIII of tables).	0.3304	
IV. Observation on adult patient with primary pernicious anæmia (Case XII of tables).	0.44	Molecular weight of volatile acids = 71. Molecular weight of propionic acid = 72.
V. Observation on adult with primary pernicious anæmia. Very excessive intestinal putrefaction (Case III of tables).	0.23	Molecular weight of volatile fatty acids = 89.
VI. Observation on adult patient with excessive intestinal putrefaction (saccharo-butyric) and slight anæmia. Mixed diet, somewhat restricted.	0.57	Molecular weight of volatile fatty acids = 71.
VII. Observation on child aged 5, with chronic intestinal putrefaction (large belly type). Putrefactive products very excessive. Saccharo-butyric type. Moderate anæmia.	0.60	Molecular weight of volatile fatty acids = 85. Molecular weight of butyric acid = 84.

In this table are seen extreme values for the volatile fatty acids of the feces. In Observation I we are dealing with a normal child; in Observation VII, with a child with an extreme condition of intestinal putrefaction and there is good reason to think that the acid values for adults fluctuate rather widely and that they sometimes reach rather high figures temporarily in persons whose health is not greatly impaired. The three observations relating to primary pernicious anæmia do not reveal extremely high values for the acids, but they are in excess of what is generally found in healthy persons on similar, somewhat restricted diets. Perhaps one reason why the differences in the acid contents of the feces in health and disease are not more pronounced is because of the free absorption of these very soluble products, which are made to some extent even in normal digestion. It seems probable that the differences in acid production in health and disease are considerably greater than the above recorded observations would indicate. It should be noted that in Observation IV, the molecular weight of the acids corresponds closely to that of propionic acid, while that of Observation VII corresponds to butyric acid and that of Observation VI falls between the molecular weights for butyric and valerianic acids. Some experiments were made to determine whether the acid production of the mixed fecal bacteria is greater when these bacteria are obtained from persons with excessive intestinal putrefaction than when they are derived from normal persons. The results were not uniform. In some instances the volatile fatty acids were greater in amount in the case of the flasks inoculated from the putrefactive cases than in the case of any inoculated from normal persons. The sediment in these cases contained large numbers of strictly anaerobic putrefactive bacteria such as B. putrificus or B. aerogenes capsulatus and both organisms were sometimes present. Peptone bouillon was employed as a medium and B. aerogenes capsulatus grew freely in this only under special conditions. The failure to find a large production of volatile fatty acids does not therefore mean that anaërobic putrefactive bacteria have been absent from the feces but may mean merely that they were unable to grow under the given conditions. B. aerogenes capsulatus usually grows well in blood bouillon (rabbits' blood may be used) and

it would have been better if this medium had been employed.

A feature of interest in this connection is that the molecular weights of the fatty acids made by the bacteria from putrefactive cases tend to be higher than the molecular weights of the fatty acids derived from the action of flora derived from relatively non-putrefactive digestive tracts. Thus the volatile fatty acids from the flora of a case of excessive intestinal putrefaction gave a molecular weight of 73; the same weight was given by the acids obtained from the flora of a case of pernicious anæmia. Another case of excessive putrefaction gave bacteria which made acids with a molecular weight of 74. These results were all obtained on sugar-free media. On the other hand the flora from a child showing extremely low putrefactive processes generated acids having a molecular weight of 64.7 in peptone medium, and 61.5 in peptone lactose (molecular weight of acetic acid being 60).

These and similar observations accord with the contention of Rodella[1] that anaerobic putrefactive bacteria tend to make the higher fatty acids. It was found also that a culture of B. putrificus (Bienstock) on peptone gave acids with a molecular weight of 86.8. A culture of B. aerogenes capsulatus (B. Welchii) gave acids which, expressed in terms of propionic acid, calculating from the weight of the barium salts, amounted to 0.257 gram per 100 c.c. of culture; expressed as a mixture of acetic and butyric acids, the yield was equivalent to 0.104 gram acetic and 0.153 gram butyric acid.

Sulphur Compounds.

Observations on the fresh feces from persons with "primary" pernicious anæmia have failed to show any peculiarities in respect to the presence either of mercaptan or hydrogen sulphide. Hydrogen sulphide could usually be detected but was not usually present in considerable amount. Repeated and careful tests (isatin-sulphuric-acid method) for the presence of mercaptans in no instance led to a positive result. This is of special interest in view of the fact to be emphasized later, that it is common

[1] "Sur la différentiation du 'Bacillus putrificus' (Bienstock) et des bacilles anaérobie tryptobutyriques (Achalme)," *Ann. de l'Inst. Pasteur*, xix, p. 804, 1905.

for the fecal bacteria from persons with pernicious anæmia to make mercaptan when grown on sugar-free peptone bouillon.

The Hydrobilirubin Reaction of the Feces.

A not unimportant indication of unusual conditions of bacterial activity in the intestine is an excessive hydrobilirubin reaction of the feces. This reaction, first described by Schmidt in 1895, is developed when one acts on certain kinds of feces in the fresh state by means of a concentrated watery solution of mercuric chloride. The marked and characteristic red color with yellowish fluorescence which appears under these conditions is believed to depend on a combination between the mercuric salt and hydrobilirubin (perhaps identical with urobilin). An analogous combination exists in Jaffe's zinc-chloride-urobilin compound. Both substances give the same spectroscopic picture—namely a band between the lines *b* and *E*.

In the course of a systematic examination of the feces from normal individuals and from many different pathological sources, it was noticed that the mercuric chloride reaction was strongest in persons suffering from intestinal disorders, especially in those with excessive intestinal putrefaction. The weakest reactions were found in the case of acholic stools and in the case of children and young adults presenting only slight indications of intestinal putrefactive decomposition (low ethereal sulphates, absence of indican and low phenol). The results in the anæmia cases are briefly summarized in the following table:

TABLE RELATIVE TO THE HYDROBILIRUBIN REACTION OF THE FECES

Case	Date of Examination	Degree of Reaction with $Hg\,Cl_2$	Behavior of Material on Exposure to Air
I	Mar. 30, '06	Very strong	
		Strong	
	Apr. 1, '06	Strong	
II	Mar. 21, '06	Very strong	Greenish; shows red-brown layer on exposure
	Mar. 28, '06	Very strong	Light brown; red layer on exposure
III	Mar. 20, '06	Very strong	
	Apr. 30, '06	Very strong	
		Almost immediately	

Case	Date of Examination	Degree of Reaction with $Hg\,Cl_2$	Behavior of Material on Exposure to Air
IV	Dec. 18, '05	Strong	
	Jan. 5, '06	Strong	
VI	Mar. 17, '06	Strong	
	Mar. 26, '06	Moderate	Greenish; reddish on exposure
VII	Jan. 18, '06	Moderate	
	Apr. 21, '06	Faint	
VIII	Dec. 30, '05	Moderate	
	Jan. 9, '06	Moderate	
	Apr. 14, '06	Very strong	
X	Nov. 28, '05	Very strong	
	Dec. 2, '05	Very strong	
	Dec. 9, '05	Very strong	
	Dec. 13. '05	Strong	
	Dec. 18, '05	Strong	
	Feb. 1, '06	Moderate	
	Mar. 14, '06	Negative	Greenish with yellow streaks
XI	Dec. 19, '05	Strong	Yellow, changing to red on exposure
XII	Dec. 27, '05	Moderate	Turns reddish yellow on surface
	Jan. 2, '06	Strong	Light yellow; turns reddish on surface
	Jan. 12, '06	Moderate	
	Feb. 1, '06	Moderate	Turns reddish-brown
	Feb. 14, '06	Strong	Turns reddish-brown
	Mar. 17 '06	Strong	
	Apr. 5, '06	Very strong	
	Apr. 27, '06	Very strong	
XIII	Apr. 17, '06	Very faint Doubtful	
XIV	Jan. 4, '06	Moderate	Dull yellow; darker on surface
	Jan. 10, '06	Moderate	

Case	Date of Examination	Degree of Reaction with $HgCl_2$	Behaviour of Material on Exposure to Air
XV	Mar. 30, '06	Strong	Very light yellow, darkening on exposure
	Apr. 30, '06	Fairly strong	
XVII	May 5, '06	Intense immediately	

The table shows that in our cases of advanced anæmia it was usual to meet with a strong mercuric chloride reaction of the feces. This result is so frequent among cases of this kind that it becomes noteworthy. Very strong reactions were observed in some cases of moderate anæmia in which putrefactive decomposition in the intestine was extreme. The reaction doubtless depends on the reduction of bilirubin in the course of putrefactive decomposition in the intestine and may therefore be expected to run parallel to the reducing activity of the intestinal bacteria. A careful comparison of the reducing activity of the intestinal bacteria, as measured by the effect of the mixed fecal bacteria on various media colored with methylene blue, neutral red, and methyl violet, failed to demonstrate any close relationship of this kind, although in general the strongest mercuric chloride reactions were found in those cases in which the intestinal reduction was most active. There were, however, cases in which the bichloride reaction was feeble despite the fact that the intestinal bacteria were shown to be capable of reducing strongly. It seems likely that another factor is necessary to make possible a strong mercuric-chloride reaction, namely the presence of a sufficient supply of biliary coloring matter in the large intestine. For it is true that when the bile is cut off from the intestine, as in obstructive jaundice, the bichloride reaction may wholly fail and is usually feeble. The persistence of the reaction in slight degree, in some cases where autopsy has demonstrated the presence of complete obstruction of the biliary duct, has been referred by some writers to a slight secretion of biliary coloring matter through the intestinal walls, and this is perhaps the true explanation. If the presence of biliary coloring matter is essential to the production of the bichloride reaction, one would expect this reaction to be particularly intense when blood destruction is from any cause excessive and permits the escape

of exceptionally large quantities of bilirubin into the intestine. The mere introduction of an excess of bile pigment into the intestine does not, however, suffice to intensify the reaction, as I have satisfied myself by means of experiments on dogs. Apparently two factors are concerned in determining the formation of hydrobilirubin—the presence of a sufficient amount of bilirubin and the existence of conditions of bacterial activity permitting the rapid reduction of this coloring matter. Outside the body the reduction of bilirubin to hydrobilirubin by bacteria was a slow process in the experiments which I have thus far made.

In some cases the feces which gave a strong hydrobilirubin reaction showed a spontaneous and marked alteration in color on exposure to the air.

The relation of the hydrobilirubin reaction to the urobilinogen reaction of the feces with Ehrlich's aldehyde is a point of interest on which one cannot at present express an opinion. As already mentioned there are instances in which the feces, after distillation[1] of all the indol present (as shown by the aldehyde reaction, which is very delicate), still give a reaction *in the cold* with a dimethylamidobenzaldehyde solution. This reaction is believed to depend on the presence of urobilinogen. It has been fairly well marked in some of our anæmia cases but there is as yet no evidence that the reaction bears any definite relation to the hydrobilirubin reaction although the two substances urobilinogen and urobilin (hydrobilirubin?) are closely related chemically.

The Ethereal Sulphates.

It is generally admitted by physiological chemists that the ethereal sulphates of the urine are perhaps the best single index to the extent of putrefaction in the intestine, or, more accurately stated, to the degree of absorption of putrefactive products into the circulation. Physiologists differ, however, as to what constitutes an excessive excretion of ethereal sulphates, some laying stress on the absolute quantities excreted daily, others attaching especial importance to the ratio between ethereal and preformed sulphates. From a long experience in dealing with this question

[1]This distillation should be conducted in an atmosphere of carbon dioxide in order to prevent oxidation of the reacting substance which is sensitive both to air and to the action of sunlight.

I have learned to emphasize especially the necessity of considering the ratio of ethereal and preformed sulphates. It has been urged against this ratio that as the preformed sulphates are derived from proteid metabolism whereas the ethereal sulphates represent putrefactive decomposition, we are comparing incommensurate things when we use the ratio as a measure of putrefaction. It should, however, not be overlooked that in health, and still more in disease, the quantity of the putrefactive products bears a relation to the quantity of proteid ingested—an excess of proteids in the dietary being the most certain means of increasing intestinal putrefaction. Against the use of the absolute ethereal sulphate value as an index of putrefactive decomposition is the fact that in health this value fluctuates widely. If the exact conditions of diet and absorption were known, this absolute value would be useful but it is ordinarily quite impracticable to obtain the necessary data. On the other hand, I have been able to satisfy myself that when the ratio of ethereal and preformed sulphates falls below 10 one almost always meets with excessive quantities of phenol or indol in the feces. It will be seen from the table that the ratio of ethereal and preformed sulphates tends to run low in the anæmia cases studied—the tendency being very pronounced in Cases II, III, IV, VIII, IX, X, XII, and XV. These results are simply corroborative of the general proposition that the putrefactive processes were excessive in the intestinal tracts of the patients under consideration.

Hunter in his well-known work on pernicious anæmia has recorded results obtained from the study of the excretion of the ethereal sulphates in a case of pernicious anæmia.[1] The results obtained in this case are similar to those reported here. The absolute quantities of ethereal sulphate are not strikingly high, but on most occasions the ratio of preformed to combined sulphuric acid is high. Hunter concludes from his figures that the absolute amount of putrefaction occurring within the intestinal canal was not excessive, but that in proportion to the quantity of proteid food ingested putrefaction was distinctly excessive. He believes further that the relative putrefactive excess was not sufficiently great to be credited with being the

[1] *Pernicious Anæmia: Its Pathology, Septic Origin, Symptoms, Diagnosis, and Treatment.* London, 1901.

TABLE SHOWING EXCRETION OF ETHEREAL SULPHATES.

Case	Date	Preformed Sulphuric Acid. Grams	Combined Sulphuric Acid. Grams	Ratio
I	Mar. 23, '06	0.6675	0.2715	9.8
	Apr. 2, '06	2.0731	0.1289	16.0
II	Mar. 21, '06	0.7421	0.1720	4.2
	Mar. 25, '06	0.2952	0.0872	3.4
III	Mar. 21, '06	0.3091	0.0580	5.3
	Apr. 27, '06	1.1124	0.2816	3.9
IV	Dec. 18, 05	0.3005	0.1444	2.1
	Jan. 5, '06	0.5891	0.1080	5.4
VI	Mar. 20, '06	0.4615	0.1522	3.0
	Mar. 24, '06	0.5846	0.0462	11.6
VII	Mar. 15, '06	0.7947	0.1167	6.9
VIII	Jan. 9, '06	0.1265	0.0210	6.0
IX	Dec. 4, '05	0.1753	0.0353	5.0
X	Nov. 27, '05	0.9930	0.2220	4.4
	Nov. 30, '05	1.5075	0.1665	9.0
	Dec. 4, '05	1.4510	0.1050	13.9
	Dec. 7, '05	0.6831	0.0809	8.4
	Dec. 11, '05	1.368	0.1317	10.4
	Jan. 31, '06	0.6234	0.1164	5.4
	Mar. 14, '06	2.7538	0.3042	9.0
	Mar. 27, '06	1.5738	0.1972	8.0
XII	Dec. 26, '05	1.1677	0.1093	10.7
	Jan. 15, '06	0.8966	0.1680	5.3
	Jan. 31, '06	0.7063	0.1066	6.6
	Feb. 16, '06	0.7565	0.1115	6.8
	Mar. 3, '06	1.1764	0.1186	9.9
	Mar. 16, '06	1.236	0.0749	16.5
	Apr. 5, '06	1.4218	0.1092	13.0
	Apr. 27, '06	1.0859	0.0691	15.7
XV	Mar. 30, '06	1.2254	0.3046	4.0
	Apr. 4, '06	1.5867	0.3313	4.7

cause of the special symptoms of so well-marked a disease as pernicious anæmia. This latter conclusion is one to which I should not wish to commit myself on the grounds brought forward by Hunter, for while, as already stated, the ethereal sulphates are the best single index of the degree of intestinal putrefaction they give no clue to the character of the putrefactive products, on which the pathological significance of the putrefaction may in an important measure depend.

On the Production of Methyl Mercaptan and of Gas by the Mixed Fecal Bacteria from Advanced Cases of Anæmia.

As the character of physiological activities of the bacteria of the feces is obviously a matter of interest in any study of the flora of the digestive tract, and as these activities can hardly be pictured with success by any method involving the formation of a composite picture through the fusion of our conceptions of the physiological activities of individual bacterial species, an attempt has been made to learn something through the cultivation of the mixed bacterial flora on artificial media. These studies relate to the production of gas, ammonia and other bases, acids, indol, phenol, mercaptan, and hydrogen sulphide. It is desired to call attention here to only two manifestations of bacterial activity—the ability to make methyl mercaptan and the capacity to produce gas. These subjects have already been touched on in a preliminary way in this Journal[1] and the methods employed have been described with sufficient fulness.

The chief drawback to the use of this method in the study of intestinal disorders is that we have no guarantee that the mixed fecal flora introduced by inoculation into an artificial culture medium will develop there in the same way as in the intestine. Indeed it is certain that in many instances the dominant types of bacteria in the culture medium are not the same as in the feces. Still the method has a certain value for the reason that the decompositions observed bear a definite relation to the flora present in the digestive tract, although not one expressive of the exact conditions of putrefaction within the body.

[1]This Journal, i, p. 415 and p. 421, 1906.

The Production of Methyl Mercaptan by Fecal Bacteria from Persons with Advanced Anæmia.

What has been learned from a study of the mercaptan reaction in relation to the anæmia cases may be briefly summarized. In seven of the cases (Nos. I, IV, IX, X, XII, XV, and XVII) strong or intense reactions for mercaptan were obtained by means of the isatin sulphuric acid test. Some of these reactions are the strongest we have observed in the course of considerable experience with the method. In Case II there was apparently no tendency to mercaptan production and in some of the other cases the inclination of the fecal bacteria to make mercaptan when grown on peptone bouillon was apparently not considerable, although in many instances the observations were not sufficiently numerous to enable one to form a positive judgment.

As pointed out in a previous paper, the persistent tendency of fecal bacteria from adults to make mercaptan on a simple peptone medium is probably to be regarded as a pathological rather than a normal manifestation, although it is true that one sometimes finds apparently normal adults whose bacteria make enough mercaptan under the described conditions to give a fair isatin reaction. Especially in the case of bottle-fed babies have I frequently found instances in which the intestinal flora were capable of making mercaptan. A slight mercaptan production may give place to the production of larger quantities with the onset of a febrile disease. The fecal flora may produce mercaptan but very little hydrogen sulphide; in general, however, the bacteria which make an abundance of hydrogen sulphide tend to make methyl mercaptan.

The explanation of the mercaptan production which has been noted in disease and sometimes in health is not yet clear. B. putrificus (Bienstock) is the only micro-organism we have yet found which is capable (in pure culture) of making mercaptan from a peptone medium. There is as yet no evidence that the observed mercaptan production by fecal bacteria is the result of the action of putrificus, but the possibility has not been definitely excluded.

I am not disposed to attach much physiological or pathological significance to the formation of mercaptan by fecal bacteria.

This is partly because experiments on dogs with high enemata of methyl and ethyl mercaptan solutions repeated daily over long periods (25 to 50 c.c. of a one per cent. solution) have

TABLE SHOWING INTENSITY OF MERCAPTAN REACTION IN PEPTONE MEDIUM INOCULATED WITH FECAL BACTERIA FROM PERSONS WITH ADVANCED ANÆMIA.

No. of Case	Date	Mercaptan Reaction	Remarks
I	Feb. 5, '06	Moderate	Reaction completed in 10 min.
	Mar. 23, '06	Strong	" " "
	Mar. 24, '06	Intense	" " "
	Mar. 27, '06	Intense	" " "
	Mar. 31, '06	Strong	" " "
	Apr. 3, '06	Intense	" " "
	Apr. 6, '06	Strong	" " "
II	Mar. 22, '06	Negative	No development in 40 min.
	Mar. 27, '06	Faint trace	Reaction occurred in 15 min.
III	Mar. 21, '06	Faint trace	Reaction required 30 min.
	May 1, '06	Negative	No development in 20 min.
IV	Dec. 18, '05	Strong	Reaction required 5 min.
	Jan. 3, '06	Strong	Deep green after 10 min.
V	Jan. 26, '06	Moderate	
VI	Mar. 17, '06	Negative	Time allowed was 30 min.
	Mar. 26, '06	Strong	Time required was 7 min.
VII	Jan. 18, '06	Negative	Time allowed was 31 min.
	Mar. 15, '06	Trace	
	Apr. 22, '06	Strong	Time, 15 min.
VIII	Dec. 30, '05	Negative	3 days' growth
	Jan. 9, '06	Trace	
	Apr. 15, '06	Intense	Reaction occurred in 10 min.
IX	Dec. 4, '05	Strong	Olive green in 5 min.

No. of Case	Date	Mercaptan Reaction	Remarks
X	Nov. 30, '05	Strong	Time allowed was 5 min.
	Dec. 8, '05	Slight	
	Dec. 12, '05	Strong	
	Dec. 18, '05	Slight	
	Feb. 1, '06	Strong	Time allowed, 5 min.; culture two days old.
	Feb. 5, '06	Strong	
	Mar. 14, '06	Strong	Time was 10 min.
XII	Dec. 25, '05	Trace-strong	Trace appeared in 5 min., became strong in 30 min.
	Jan. 2, '06	Strong	Culture 1 day's growth
	Jan. 2, '06	Negative	2 days' growth in cystin medium
	Jan. 17, '06	Negative	
	Feb. 1, '06	Strong	Time, 5 min. on culture 4 days old
	Feb. 1, '06	Negative	
	Mar. 4, '06	Trace	Time allowed, 30 min.
	Mar. 18, '06	Intense	Time allowed, 7 min.
	Apr. 6, '06	Strong	Time allowed, 30 min.
	May 1, '06	Intense	Time, 10 min.
XIII	May 1, '06	Moderate	Time allowed, 50 min.
XIV	Jan. 3, '06	Negative	
	Jan. 10, '06	Negative	
	Feb. 5, '06	Intense	Reaction developed in 5 min. in a culture 2 days old.
XV	Mar. 30, '06	Strong	Time allowed, 10 min.
	Apr. 6, '06	Strong	Time allowed, 25 min.
	May 1, '06	Negative	Time allowed, 20 min.
XVI	Dec. 1, '05	Trace	Time allowed, 20 min.
	Dec. 8, '05	Fairly strong	
	Dec. 15, '05		
	Dec. 22, '05	Strong	Time allowed, 10 min.
XVII	May 5, '06	Intense	Time allowed, 15 min.
	May 7, '06	Intense	Time allowed, 10 min.

failed to induce definite toxic manifestations. Moreover it appears to me by no means certain that methyl mercaptan is actually formed in the intestine (and absorbed therefrom)

in appreciable quantity by the bacterial flora which, outside the body, have been found able to make this sulphur compound. Although hydrogen sulphide is readily detectable in the freshly passed feces of many individuals with advanced anæmia, I have never been able to detect more than a trace of methyl mercaptan even in quite fresh material. This fact, though not conclusive, makes one question whether mercaptan is formed in any part of the intestinal tract in quantities that possess any pathological significance.

But notwithstanding the absence of evidence that mercaptans are factors in the production of intoxications, the ability of certain flora to make these substances outside the body, on a peptone medium, is of some biological interest and it has seemed desirable to state here the persistence with which mercaptan formation occurs under the action of bacteria derived from the intestines of certain anæmic patients.[1]

On the Restricted Formation of Gas by the Fecal Flora Grown on Sugar Bouillon.

Early in the course of the present investigation it was noticed that the fecal flora from certain individuals almost regularly fall far below the normal standard of activity in gas production on sugar-bouillon media. It was observed also that material derived from persons with pernicious anæmia and allied blood diseases is so apt to show the peculiarity of low gas production that it must be regarded as a feature of such affections, although by no means limited to them.

As already explained elsewhere[2], the mixed fecal flora from healthy adults on a mixed diet make considerable gas when grown on sugar bouillon for twenty-four hours, and the volume of gas made under fixed conditions is apt not to vary widely in the case of the same individual, if the dietetic conditions remain similar. In the observations made in my laboratory, the practice has been followed of inoculating four sugar-bouillon fermentation

[1] Dr. Rettger writes me that he has obtained a strong mercaptan reaction from the products of growth of the mixed fecal bacteria from three patients with pernicious anæmia. I have also observed another case of pernicious anæmia (not included in the above report) in which a very strong reaction was noted.

[2] This Journal, i, p. 415, 1906.

tubes (dextrose-, levulose-, lactose-, and saccharose-bouillon) with the material to be tested. Usually the gas production has been greatest in the lactose tube and least in the saccharose tube. The column found in each tube was measured and the total gas production in the series of four tubes was recorded. It was found that in normal individuals the average gas production for the four tubes is about 100 millimeters.[1] Among healthy individuals on a mixed diet, the fecal flora seldom yield less than 65 millimeters of gas in the four tubes. The bacteria derived from persons with digestive disorders, on the other hand, very frequently give not more than 60–75 millimeters of gas in the four tubes. In the case of persons suffering from blood diseases, a very much greater restriction in gas production has been noted, as will be seen by reference to the table. The tendency to low gas production is especially notable in Cases I, II, VI, VII, VIII, X, XIV, and XVII. In Case XII, in which eight observations were made, the gas production fell below 73 millimeters only on three occasions (45, 60, and 66 millimeters).[2] In Case XV, also, we have three observations which fall within the normal limits (79, 103, and 136 millimeters), this case being one of secondary anæmia associated with pronounced putrefactive conditions. In looking over the table of recorded results

[1] The anaerobic limb of our fermentation tubes measures about 95 millimeters.

[2] It should be stated that after the manuscript of this paper had been sent to press the gas production by the fecal flora from Case XII fell to 25 millimeters (in the four sugar-bouillon tubes). This fall in gas production was coincident with a period of relapse in which the red blood cells and hæmoglobin declined considerably (although only temporarily). At the same time the microscopical fecal fields showed a very marked increase in bacteria of the type of B. aerogenes capsulatus and a falling off in organisms of the B. coli type. Typical B. aerogenes capsulatus was found abundantly on the highly anaerobic agar plates prepared at this time. The presence of this organism in unusual numbers was furthermore proved by the gas production noted in an incubated rabbit which had been injected with an exceptionally dilute suspension of the fecal bacteria (1–50). Moreover it was impossible to recover bacteria of the B. coli group from gelatin plates made at this period. Finally, it should be noted that the fecal bacteria grown in peptone bouillon produced methyl mercaptan in unusual abundance, judging by the intensity of the isatin reaction.

it will be seen that there are other instances in which gas production has fallen within the normal limits.

A feature of much importance to the interpretation of the recorded results is the presence or absence of diarrhœa. Repeated observations have shown that the occurrence of diarrhœa tends to increase the gas-forming activities of the fecal flora. The explanation of this fact is, I think, obvious. It has been found that the gas production, in the four sugar-bouillon tubes which has been incited by the action of pure cultures of B. coli derived from human sources resembles closely in volume and chemical character the gas production by the mixed fecal bacteria from normal adults. This fact, viewed in connection with the observed growth of organisms of the B. coli type in the fermentation tubes, has led to the inference that a large part of the gas production on the part of the fecal flora is due to the activities of organisms of the B. coli type. It is known that these bacteria tend to die as they approach the rectum from higher levels of the intestine, and that living gas-producing bacteria (B. coli and lactis aerogenes types) are more numerous in the upper than in the lower colon. It can easily be shown that conditions which induce a rapid passage of the intestinal contents through the gut bring down increased numbers of living gas-forming, Gram-negative bacteria. The relative increase in B. lactis aerogenes is probably a factor in the greater gas formation.

A comparison of the pictures presented by the Gram-stained, microscopical, fecal fields with gas production induced by the bacteria which constitute these fields, leads to one definite conclusion. It is that Gram-positive fields, containing relatively few or poorly preserved micro-organisms of the B. coli type are associated with restricted gas production almost without exception. On the other hand, fecal material showing Gram-negative fields, made up of well-preserved bacteria corresponding morphologically to the type of B. coli, rarely fail to produce gas abundantly.

The observed failure to make gas in normal amount in pernicious anæmia, I refer to a diminution in the number of living micro-organisms of the B. coli type in the feces, though there is no proof that in some instances this factor may not be reinforced by the inhibitory activity of other types of micro-organisms.

TABLE SHOWING INTENSITY OF GAS PRODUCTION IN PEPTONE MEDIUM INOCULATED WITH FECAL BACTERIA FROM PERSONS WITH ADVANCED ANÆMIA.

No. of Case	Date	Amount of Gas Production. Millimeters	Remarks
I	Feb. 5, '06	97	Diarrhœal movement
	Mar. 20, '06	(1) 26	
		(2) 10	
	Mar. 24, '06	5	
	Mar. 27, '06		
	Mar. 31, '06	54	
	Apr. 3, '06		
	Apr. 6, '06	62	
II	Mar. 22, '06	62	
	Mar. 27, '06	35	Coli type poorly preserved
III	Mar. 20, '06	62	Coli type fairly numerous; fairly preserved
	Apr. 29, '06	114	
IV	Dec. 18, '05	155	Diarrhœa
	Jan. 5, '06	45	Coli type present; preservation poor
V	Jan. 26, '06		Coli type abundant; poorly preserved
VI	Mar. 17, '06	54	Coli type only moderately abundant
	Mar. 26, '06	56	Coli type only moderately abundant
VII	Jan. 18, '06		Coli type poorly preserved
	Mar. 15 '06		
	Apr. 20, '06	43	Blood condition much improved
III	Dec. 30, '05	95	Diarrhœa
	Jan. 9, '06	70	
	Apr. 14, '06	63	

No. of Case	Date	Amount of Gas Production. Millimeters	Remarks
IX	Dec. 4, '05	160	Diarrhœal movement
X	Nov. 28, '05	25	
	Nov. 30, '05	30	
	Dec. 5, '05	100	Diarrhœal movement
	Dec. 8, '05		
	Dec. 12, '05		
	Dec. 18, '05		
	Dec. 28, '05	60	
	Feb. 1, '06	30	
	Feb. 5, '06		
	Mar. 14, '06	20	
XII	Dec. 25, '05	85	Semi-solid movement
	Jan. 2, '06	85	Movement soft
	Jan. 17, '06	112	Soft movement from high enema
	Feb. 1, '06	45	Movement formed
	Feb. 14, '06	76	Movement formed
	Mar. 4, '06	60	Partly formed movement
	Mar. 18, '06	66	Partly formed movement
	Apr. 6, '06	102	Soft, unformed movement
	Apr. 28, '06	86	Semi-solid movement
XIII	Apr. 18, '06	116	Fluid movement
XIV	Jan. 3, '06	35	
	Jan. 10, '06	38	
	Feb. 5, '06	53	
XV	Mar. 30, '06	103	Soft, unformed movement
	Apr. 4, '06	79	Partly formed movement
	Apr. 30, '06	136	
XVI	Dec. 1, '05	20	
	Dec. 8, '05	100	
	Dec. 15, '05	150	
	Dec. 22, '05	95	
XVII	Apr. 30, '06	15	Semi-solid, pasty movement
	May 4, '06	25	

The considerable or normal or even excessive gas production noted in some cases of pernicious anæmia I think may be reasonably ascribed to the presence of gas-producing organisms (of the B. coli type) incidental to diarrhœa. This is evidently true of many of the cases which have come under my observation. It is probable, however, that other bacterial influences are sometimes at work. In a few fermentation tubes in which there has been high gas production and in which organisms of the B. coli type have been scanty, there has been evidence of multiplication of organisms morphologically like B. aerogenes capsulatus (B. Welchii). This organism is a rapid and abundant gas-maker on sugar-media and may have been responsible for the gas formation in the cases mentioned. If this be the case, it shows that a normal volume of gas in the fermentation tubes does not necessarily mean that the fermentation has been carried on by the normal gas-makers of the intestine—*i.e.*, by organisms of the B. coli and B. lactis aerogenes group. Ordinarily B. aerogenes capsulatus does not grow well on sugar bouillon in the absence of blood or fresh animal tissues (such as liver) and it is not yet clear to what conditions the occasional observed growth has been due.

It is believed that the phenomenon of restricted gas production by the fecal bacteria is one of much biological and pathological interest and that the method here employed in the study of gas production will prove of clinical value. The phenomenon of restricted gas production is by no means limited to the bacteria derived from persons with severe anæmias but is observed in lesser degree in many digestive disorders.

I have several times observed it in the course of fever and think the explanation here is probably the same as in the cases of digestive derangement—*i.e.*, the partial elimination of living colon bacilli from the feces—a condition of colon scarcity.

In a recent paper which promised to prove significant for the understanding of bacterial conditions in the intestinal tract, Conradi and Kurpjuweit advanced experimental evidence to show that the obligate colon bacilli of the human intestine produce substances capable of holding in check the development of other species of bacteria,—for example the typhoid and paratyphoid organisms. To these inhibitory substances they gave

the name "autotoxines," as it appeared that the colon bacilli were themselves subject to the inhibitory influence exerted by the substances produced by them. If such inhibitory powers could have been shown to reside in the colon bacilli, it would have thrown an important light on our knowledge of the antagonisms between intestinal bacteria, and especially on the role of the colon bacillus group in helping to exclude saprophytic forms. The work of Conradi and Kurpjuweit has, however, been recently subjected to criticism and it seems clear that the phenomena of inbibition which they described as being dependent on autotoxines are open to other explanations.[1] It appears from these criticisms that the phenomena of inhibition attributed to the formation of autotoxines are probably due, at least in part, to the exhaustion of the nutrient media in which the colon bacilli have grown. Moreover the inhibition of the growth of typhoid bacilli and other bacteria appears to have been much less complete than was supposed by Conradi and Kurpjuweit. It is a matter of considerable importance in connection with the present study to know whether a free growth of the obligate colon bacilli in the intestine operates in such a way as to check the development of Bacillus aerogenes capsulatus. It is certainly true that a greatly diminished representation or a complete elimination of typical colon bacilli from the feces is a feature of many cases of severe anæmia and it is possible that this partial or complete disappearance of colon bacilli constitutes a condition especially favorable for the infection of the digestive tract by Bacillus aerogenes capsulatus.

The Examination of Sediments from Fermentation Tubes.

In speaking of the study of the Gram-stained fecal bacterial fields, some emphasis is laid on the value of this method in gaining an acquaintance with the dominant bacterial flora of the lower part of the intestine. There is perhaps no better intro-

[1] See especially the criticisms of Moro and Murath, "Ueber die Bakteriellen Hemmungsstoffe des Säuglingsstuhles," *Wien. klin. Wochenschr.* xix, p. 371, 1906; also F. Passini, "Die bakteriellen Hemmungsstoffe Conradis und ihr Einfluss auf das Wachstum der Anaerobier des Darmes," *ibid*, p. 627; and R. Oebius, "Ueber spontane Wachstumshemmung der Bakterien auf künstlichen Nährboden," *Med. Klinik*, ii, p. 598, 1906.

duction to the bacterial conditions that prevail in the intestine than the careful examination of a series of microscopical fields from representative parts of the material derived from the lower bowel. The method has, however, an important limitation which should be clearly recognized. It is that the Gram stain gives us no positive indication as to which bacteria are living and which are dead. It is important to be informed on this subject, for if the apparently dominant organisms in a field are really for the most part dead and autolysing, it is obvious that they do not constitute the physiologically dominant variety. Especially in the case of micro-organisms of the B. coli group is it desirable to have some information on this point, since these organisms probably exert a protective action against certain invaders of the intestinal tract. It is often possible to gain some knowledge as to the living and dead varieties of fecal bacteria by examining the sediments from the fermentation-tube cultures which have been employed in studying the gas production, indol production, etc., of the intestinal flora. The results of such study have developed the following facts: (1) organisms of the B. coli group may fail to grow freely in the anaerobic limb of the fermentation tube on glucose bouillon, or may not grow at all, after inoculation with fecal flora which appear by the Gram stain to contain many (though perhaps poorly preserved) organisms of the B. coli type. The inference in such cases is that living B. coli were in reality not well represented in the feces, although in some cases another possibility presents itself—namely that the bacteria of this type though not dead have been inhibited by other species. Among our patients with advanced anæmia the fermentation tube has in several instances pointed to the absence of B. coli in the fecal contents,—always in cases that have shown restricted gas production. Among normal or nearly normal persons who have been used for control observations, a failure of these organisms of the B. coli type to make gas has not been observed. In a case of diabetes, however, with Gram-positive fields, B. coli failed to grow—again with small gas production. The absence of living B. coli in the feces of some patients with pernicious anæmia is further indicated by the failure of representatives of this group to appear on litmus-gelatin plates.

(2) The positive diplococci which are normally seen in the

feces grow readily in the sugar-bouillon fermentation tubes and are apt to be well represented in the sediment both from normal and pathological cases.

(3) Streptococci are sometimes found in great abundance in the fermentation-tube sediment. This is especially apt to happen in the case of material which microscopically shows the presence of a considerable number of streptococci; but it may also happen that there is an abundant and dominant growth of streptococci in cases where the microscopical fields have failed to call attention to its presence.[1]

(4) An abundant growth of an organism having the morphological characters of B. aerogenes capsulatus was a frequent occurrence in the tubes inoculated from patients with advanced anæmias. This was observed in Cases I, II, III, VI, VII, VIII, IX, and XVI, and also in a case of diabetes, material from which had shown capsulatus-like organisms in the Gram-stained fields. On the other hand, capsulati were not ordinarily observed to grow in the tubes inoculated from the feces of normal persons selected as controls. The growth of B. aerogenes capsulatus was generally most abundant in the lactose-bouillon tubes.

Action of the Mixed Fecal Bacteria on Milk.

Among the first observations made in the course of this research was the fact that sterilized milk undergoes a peculiar "stormy fermentation" when inoculated with the mixed fecal flora from certain individuals, whereas this is not induced by bacteria from other sources. In this fermentation the casein is coagulated, broken up into small fragments, and undergoes some degree of peptonization, while there is at the same time a rapid

[1] In one instance in which the apparently normal feces contained very many leucocytes and only a few coccal forms in the fields, the fermentation-tube sediments showed the growth of streptococci only, colon organisms failing to grow in most of these tubes. It afterwards developed that the patient had a small dental abscess, containing streptococci, the pus from which had for two years been passing into the stomach. The chief clinical conditions were anæmia, mental depression, and loss of weight. I am indebted to Prof. James for the opportunity of studying these conditions. A very similar observation was made in the case of a young woman who developed mucous colitis during convalescence from measles.

and voluminous liberation of gas. The meaning of the phenomenon was not at first clear, but it now seems certain that it is referable to the action of bacteria of the B. aerogenes capsulatus type. This view is borne out by the following facts: (1) B. aerogenes in pure culture sets up the "stormy fermentation" just mentioned; (2) the fecal flora setting up this type of fermentation in milk were derived almost exclusively from persons in whose stools micro-organisms of the B. Welchii type were found in considerable abundance (including nearly all the cases of "primary" pernicious anæmia in which this clinical diagnosis appeared justified); (3) the fecal flora from persons showing slight signs of intestinal putrefaction and few or no capsulati in the feces failed to give the characteristic active fermentation of milk. These results, however, which differentiate between the action of bacterial suspensions containing few capsulati and those containing many, are obtainable only by inoculating the fermentation tubes with small quantities of the suspension. If one inoculates large quantities of the suspensions the number of capsulati contained in the normal material will sometimes be sufficient to induce the typical change in milk in the course of twenty-four hours.

BACTERIOLOGICAL OBSERVATIONS

(In Conjunction with Herbert C. Ward.)

The necessity of obtaining definite knowledge of the bacterial inhabitants of the digestive tract, in conditions attended by evidence of excessive putrefaction within this tract, is self-evident. In the case of our group of anæmias this necessity was further emphasized by the frequent recurrence, within the group, of a phenomenon, calling for a bacteriological explanation—the phenomenon, already described, of small gas production in sugar bouillon. The problems relating to the bacterial flora of the intestine are so beset with technical difficulties and difficulties of interpretation that even their proximate solution (in the sense in which any life-phenomena are soluble by scientific methods) is a task of the not very near future. What we have to offer on the subject of the bacteriology of the intestine in anæmia is put forward with a realization of its inadequacy, but in

the belief that facts thus far collected represent a necessary step in progress.

There are several methods by which we have sought to obtain knowledge of the bacterial conditions in the intestinal passages of persons with apparently primary, profound anæmia, in the hope of finding in these conditions something to account for the evidences of putrefactive decomposition and of restricted fermentation, which we have found to characterize these cases. There are three general methods of approach from which, up to the present time, we have had the most encouragement. These are: (1) the microscopical study of the Gram-stained fecal fields; (2) the microscopical study of the fermentation-tube sediments derived by inoculating certain media with the mixed fecal bacteria; and (3) the study of the bacteria cultivated under strict anaerobic conditions from fecal suspensions subjected to pasteurization. These methods and their results will be separately described and their relation to each other will be considered.

Study of the Gram-stained Fecal Fields.

The microscopical examination of the Gram-stained fecal fields (counterstained with saffranin or carbol-fuchsin) has proved in several respects a serviceable method. It enables the observer to roughly classify the bacteria of a given field as strongly positive, positive, mixed, negative or strongly negative. This is a real gain, since it gives at once a clue to the relative importance of organisms of the colon bacillus type—a feature which, as will later be made evident, is of prime importance. The recognition of the dominant bacterial types is much facilitated by this study, for it usually enables the practised observer to distinguish with a serviceable degree of accuracy organisms of the following types: B. coli, B. pyocyaneus, B. aerogenes capsulatus, B. bifidus, various diplococci and staphylococci, yeast organisms, etc. The presence of spore-bearing organisms and of free spores can also be made out. The presence of capsules can also be made apparent in some instances.

In the following tables are recorded some features of typical fecal fields from a group of anæmia patients and from a group of "normal" persons, chosen almost at random.

The features which find expression in the tables are the char-

acter of the fields with respect to the Gram stain, the abundance and state of preservation of organisms of the B. coli type, the presence of positive coccal forms, the presence of spore-bearing, capsulatus-like bacilli,[1] and the presence of oval spores, such as are under some conditions formed by B. Welchii. Other bacterial characters of the fields have been for the time being ignored. The above characteristics of the fields were selected for the following reasons. It had been noted that in certain cases the fecal flora are unable to ferment sugar bouillon with full gas production. As gas production in fermentation tubes inoculated with normal adult feces appears to be mainly due to organisms of B. coli and B. lactis aerogenes type (B. Welchii not growing readily even in the anaerobic limb of the tube, on sugar bouillon without blood or animal tissues) the character of the fields with regard to B. coli became a question of interest. Negative fields with an abundance of well-preserved organisms of B. coli type one would expect to see in those feces that give an abundance of gas; positive fields with relatively few coli forms in a poor state of preservation one would expect to find associated with deficient gas production on sugar bouillon. Both these relations have been found to hold true in general. Attention was called to the importance of positive cocci by observation on the sediments of fermentation tubes in which both cocci and B. coli had been grown. The cocci clearly tend to retard the gas-producing action of B. coli on sugar bouillon. This is a result in harmony with the similar observations lately made by Heinemann[2] in another connection. The reason for paying special attention to the frequency of organisms of the B. Welchii type is that on beginning the study of the strict anaerobes from an anæmia patient conjointly studied with Professor Theobald Smith, it was found that there occurred regularly on the blood-agar plates large

[1] I have come to believe that these Gram-positive, spore-holding bacilli, which are morphologically indistinguishable from B. aerogenes capsulatus do not as a rule belong to this class but to a group of aerobic and physiologically very different class of organisms. The reasons for this opinion will appear later.

[2] "The Significance of Streptococci in Milk," *Journ. of Infect. Dis.*, iii, p. 173, 1906. It is here pointed out that B. aerogenes var. lacticus is held in check and ultimately stopped by the presence and ascendency of streptococcus lacticus.

numbers of this organism and extremely few other strict anaerobes of the spore-forming class.[1] It was shown by Hirshberg[2] in Professor Welch's laboratory that B. aerogenes capsulatus is a very frequent inhabitant of the intestinal tract of man and other animals[3] and it had been shown by Welch[4], Howard,[5] and others, that local lesions of the gastro-enteric tract occur which are attributable to this organism. It therefore appeared desirable to inquire more closely than had been done into the occurrence of B. Welchii in the intestinal contents in health and disease. There soon appeared indications that there are numerous instances in which B. Welchii is very abundant (as compared with its occurrence in normal individuals) in the feces. The study of the question from the standpoint of its occurrence in persons with advanced apparently primary anæmias assumed increasing interest and the results on the fecal fields thus far noted are embodied in the table.

If we compare the table of normals with that of the anæmias the following differences may be noted: (1) the tendency of the normal fields is toward Gram-negativeness while that of the anæmia fields is toward Gram-positiveness; (2) the representation and preservation of B. coli are poor in most of the anæmia fields as compared with the representation of this group in the normal fields[6]; (3) positive coccal forms are distinctly more abundant in the anæmia group than in the normals (in one of the cases [VII, March 15] positive diplococci were extremely abundant

[1] Our attention was originally and previously directed to B. putrificus (Bienstock) as a cause of excessive intestinal putrefaction, but this line was abandoned because it appeared unpromising.

[2] "Distribution of Bacillus Aerogenes Capsulatus (Bacillus Welchii, Migula)," *Journ. Bost. Soc. for Med. Sci.*, v, p. 369, 1900–1901.

[3] We have found that the feces of tigers may consist almost wholly of free spores at least a portion of which develop into the vegetative form of B. aerogenes capsulatus.

[4] *Bull. Johns Hopkins Hosp.*, Sept., 1900.

[5] *Contributions to the Science of Medicine Dedicated by his Pupils to William Henry Welch*, Baltimore, 1900, p. 461.

[6] It is often difficult to form a positive judgment from the microscopical appearances as to the condition of B. coli in the fields, but with practice it becomes possible to predict in many instances the behavior on culture media.

and made up the dominant bacterial type); (4) bacilli of the type of B. Welchii are distinctly more abundant in the anæmia fields than in the normal ones, the difference in this respect between the two sets of cases being pronounced; (5) spore-bearing organisms of the capsulatus type were rarely seen in the normal fields and were not a feature in the anæmia cases, although in three instances (Cases I, V, and X) they were present; (6) free oval spores, suggesting capsulatus spores, were not usually abundant either in the normal cases or in the anæmias, but in three of the latter cases (Cases I, V, and X, the cases showing spore-holding bacilli) they were numerous and often were seen in clumps and chains.

It is especially important to note that there are four instances (Cases VII, X, XV, and XVI) in which the fields showed an abundance of B. aerogenes capsulatus when the patients first came under observation and that at a later period, after treatment was begun, there was in these same cases a noteworthy falling off in their number. At the same time there was noted in three of these instances an opposite change, *i.e.*, an increase, in regard to negative bacteria of the B. coli type. The observations included in the table which illustrate the alterations in the bacterial flora are in harmony with observations on other material from the same cases which have not been included in the table.

It is desirable to state what is meant when speaking of the 'capsulatus type" in the fecal fields. The term is used to refer to rather large, plump, Gram-positive bacilli, usually with slightly rounded free ends and square-cut opposed ends, sometimes showing capsules—organisms morphologically typical of B. Welchii. They frequently occur in pairs, end to end. More frequently they occur singly. Occasionally they appear as long threads. The term is here further used to include bacilli somewhat smaller and thinner than the typical B. Welchii, but having in other respects the same morphological characters and the same staining properties. These latter organisms, which are sometimes numerous in the fields, are included in the "capsulatus type" because it is known that under certain conditions the true Bacillus aerogenes capsulatus assumes these characters and because our more detailed studies of colonies

of this sort have shown them to be abundant gas producers and otherwise to conform to the characters of B. aerogenes capsulatus. There is, however, another Gram-positive organism, smaller than that just mentioned, and uncapsulated, which is less strictly anaerobic than B. Welçhii and makes little or no gas, which must be carefully distinguished from the "capsulatus type." It has not yet been fully studied by us, but probably corresponds to the plain forms of Tissier's B. bifidus. Furthermore it seems wise at present to speak of the capsulatus type, in referring to the fecal fields because there is reason to believe that there are sub-varieties of Welch's gas bacillus, sub-varieties based mainly on differences respecting the difficulty of sporulation, upon pathogenic qualities, hæmolytic properties, indol production, rapidity of gas production in animals, etc.

Finally it must be stated that there is sometimes found in the feces an organism morphologically so similar to the true B. aerogenes capsulatus as to be indistinguishable from it.[1] It is evident

[1] The pseudo aerogenes organism which is here in question possessed the following characters in a case where it had been isolated from the feces of a person suffering from excessive intestinal putrefaction. The organism did not differ in any appreciable way in size or morphology from typical B. aerogenes capsulatus. It was Gram-positive and proved to be a facultative anaerobe capable of abundant growth aerobically in peptone bouillon. On ordinary fluid media, it formed a pellicle on the surface which after a time fell to the bottom. It was found to sporulate readily after a few days' growth on agar. It was sluggishly motile. The organism produced no gas on dextrose, levulose, lactose, or saccharose bouillon. It grew luxuriantly on agar slants and plates forming a dense, opaque, white, dry, and wrinkled surface. It produced acid on dextrose and saccharose. On peptone bouillon it produced neither hydrogen sulphide nor mercaptan nor indol nor skatol. It produced volatile fatty acids, apparently chiefly butyric acid, after several days' growth on peptone bouillon. It also produced ammonia. Two cubic centimeters of the bouillon culture ineculated into the femoral vein of a rabbit produced no gas and failed to give rise to the very characteristic decomposition induced by B. aerogenes capsulatus. In this experiment the organism in a state of involution was found in moderate numbers in the spleen but it had failed to grow in the liver. Two cubic centimeters of the bouillon culture injected intraperitoneally into a guinea-pig gave rise to no symptoms. The organisms had no hæmolytic action upon blood nor did they show any capacity to reduce hæmoglobin. The spores formed by this aerobic organism were oval and were located midway between the middle and end of the bacillus. No capsule was observed when the organism was grown on ordinary media.

from this fact that we cannot safely rely upon the appearance of the Gram-stained fecal fields as a certain index of the presence of B. aerogenes, although it appears probable that in most cases in which the feces show an abundance of bacilli suggesting this organism the true B. aerogenes capsulatus is in reality present in numbers pointing to an unphysiological condition of the bacterial flora.

The Growth of Micro-organisms of Capsulatus Type on Sugar-Blood-Agar Plates under Anaerobic Conditions.

Perhaps the most satisfactory method of determining the presence of organisms of the capsulatus type in the feces is that which consists in growing these organisms on sugar-blood-agar plates. This method not merely gives evidence as to the presence of the bacteria in question, but can be utilized to gain a rough idea of the numbers present in the feces. In the present instance no effort was made to get an idea of the actual numbers of capsulatus present in the feces, but the suspensions of fecal material used for inoculating the sugar-agar plates were so employed as to give a fair basis for comparison of the numerical results in pathological and normal conditions.[1] The results here given relate to the spores and spore-bearing varieties of bacteria in the feces and not to vegetative forms, for the suspensions containing the intestinal flora were subjected for twenty minutes to a temperature of 80° C. in order to eliminate vegetative forms of bacterial life. In each case duplicate series of plates were made, one series being incubated under aerobic conditions, the other under anaerobic conditions. A satisfactory state of anaerobiosis within the plates was obtained with the aid of a hydrogen stream from gas stored in the compressed form in tanks. The pyrogallic acid method of securing absorption of oxygen was employed as an auxiliary procedure to get rid of the last trace of oxygen and to indicate the conditions within the bell-jar.

The results obtained in this way are given in the accompanying

[1] The fecal suspensions were made in approximately the same way in the normal and pathological cases, and the inoculation of the agar plates was made with quantities of the fecal suspension designed to avoid an excessive number of anaerobic colonies on the plates.

tables, where the presence of aerobic, spore-bearing organism is indicated in addition to the presence of anaerobes. This double procedure is of value because it has occasionally revealed the presence of an organism resembling capsulatus in morphology, and which makes oval spores, but grows aerobically as well as anaerobically and does not make gas in the manner characteristic of the true B. aerogenes capsulatus.[1]

Inspection of the tables shows clearly the difference in results yielded by material from normal persons as compared with that derived from our advanced anæmias. The number of organisms of the capsulatus type found on the anaerobic plates made from material from persons with normal digestion—that is with little indication of putrefactive decomposition—is small where such organisms were found at all. One of the persons selected as a control showed a considerable number of capsulatus colonies (about 100 anaerobic colonies being found on the plate) but it should be noted that in this individual putrefactive disturbances are of frequent occurrence and are readily induced by slight errors in diet. A considerable number of capsulatus-like organisms were observed on the plates made from an adolescent patient with advanced diabetes and signs of impending coma. In a number of instances the plates from the controls showed no colonies suggestive of capsulatus. Plates made from the anæmia cases, on the other hand, almost without exception showed the presence of capsulatus-like colonies and often these were numerous. In one of the cases observed by us a distinct fall in the number of capsulatus-like organisms on the plates was observed as the patient convalesced and the same observation was quite independently made on this patient by Professor Theobald Smith. Unfortunately the conditions did not permit us to study all our cases in this manner for in many instances they came under notice only after treatment had been commenced and the original blood picture had been altered.

In conclusion it may be said that the plate method has confirmed the observations already made from the study of the Gram-stained fecal bacterial fields, that organisms of the capsulatus type occurred in much greater abundance in the feces of persons with advanced anæmia than in those of normal persons.

[1] The characters of this organism are given on page 39, footnote.

differences in the number of capsulati habitually present in the case of different individuals. There are some young persons between the ages of five and twenty years from whom it is either very difficult to obtain B. aerogenes capsulatus by plating or whose movements give no evidence whatever of its presence. If we prepare a suspension of the feces from such individuals by grinding 1 gram of the fresh material with 9 c. c. of 0.85 per cent. salt solution and filtering through absorbent cotton, we can inject intravenously one or two cubic centimeters of this suspension into a rabbit and then incubate the quickly killed rabbit for five hours at 37° C. without obtaining evidence of the abundant presence of the gas bacillus. On opening a rabbit which has been thus incubated, one finds none of the signs of the activity of the gas bacillus—no accumulation of gas in the liver or vessels or in the connective tissues or serous cavities. Moreover smears made from the liver blood or the auricular blood either do not show the presence of capsulatus at all or these organisms are present only in small numbers. If, however, the foregoing experiment be made with the fecal material derived from a patient with pernicious anæmia or from a person suffering from a capsulatus diarrhœa, one generally gets an entirely different result. At the end of five hours the liver is soft and friable, crepitates between the fingers, and on section shows the presence of many bubbles of gas. There may also be a small accumulation of gas in the peritoneal cavity. Smears from the hepatic blood and from the auricular blood swarm with organisms of the capsulatus type. The spleen also contains such organisms in great numbers.

The following protocols are instructive in this connection.

Experiment 1. One c.c. of a filtered fecal suspension, from a normal person 16 years of age, almost free from putrefactive products in the urine, was injected into a rabbit which was immediately killed by a blow on the neck. After five hours' incubation, the animal was examined. Abdomen distended slightly from distension of large intestine; liver firm, slightly friable and free from gas. A stained liver smear shows a few short bacilli (not capsulatus). Heart's blood shows short bacilli in abundance, rarely a bacillus of capsulatus type.

Experiment 2. Two c.c. of filtered fecal suspension from a healthy man (æt. 42) recently recovered from universal eczema, were injected intravenously into a rabbit which was then promptly killed by a blow on the neck. Animal incubated for 5 hours at 37°C. On examination, no

odor of butyric decomposition; liver firm and without gas bubbles. Smear from heart's blood showed a few bacteria of capsulatus type.

Experiment 3. Two c.c. of filtered fecal suspension from a healthy breast-fed baby were infused intravenously into a rabbit which was then promptly killed. Examination after 5 hours' incubation at 37° C. reveals no odor of butyric putrefaction and liver is firm and free from gas. Smears from the heart's blood show it to be free from bacteria of any kind.

These experiments may be contrasted with the following:

Experiment 4. Two c.c. of filtered fecal suspension from an anæmic baby with irregular diarrhœa (Case XVI of tables) were infused intravenously into a rabbit which was then promptly killed and incubated for 5 hours at 37° C. On examination the tissues gave a strong butyric acid odor. Liver soft, friable, and filled with bubbles of gas. Smears from heart's blood show a great abundance of bacteria of the capsulatus type.

Experiment 5. One c.c. of filtered fecal suspension from a patient with pernicious anæmia (Case VII of tables) was infused intravenously into a rabbit which was then incubated for 5 hours at 37° C. On examination the abdomen was slightly distended. Characteristic butyric odor. Liver crepitant, contains a few obvious gas bubbles. Bacilli of capsulatus type abundant in heart's blood, in almost pure culture. Capsulati also abundant in liver.

Experiment 6. Two c.c. of filtered fecal suspension from a patient with pernicious anæmia (Case I of tables) whose feces contained a great abundance of free (capsulatus?) spores were injected intravenously into a rabbit which was incubated at 37° C. for 24 hours. At autopsy the animal was greatly distended with gas, and bloody fluid was oozing freely from nose, mouth, etc. Gas escaping from abdominal cavity burns with blue flame. Extremely offensive odor of butyric decomposition. Tissues in advanced state of putrefactive liquefaction. Blood from heart shows bacilli of capsulatus type to be extremely abundant. Most of these were Gram-positive and occurred characteristically in diplobacillus form, but there were also many long threads which were doubtless capsulati. Gram-negative forms also occur and of these one variety was especially prominent. This was a long organism bearing a large spore near either end. It is probably to be regarded as a young form of capsulatus about to undergo division midway between the spores. This organism was Gram-negative.

The foregoing experiments are typical of a large group and show plainly enough the difference in capsulatus activity in the case of material derived from normal and pathological feces. It appears to be true that a 10 per cent. suspension of feces does not excite an active formation of gas in the liver, if the material

Grassberger and Schattenfroh, whose granulo-bacillus saccharobutyricus immobilis liquefaciens was derived from milk and is regarded by Welch, Kamen, and others as identical with B. aerogenes capsulatus, found it to be non-toxic for guinea-pigs. Kamen[1] although unable to obtain powerful toxins on ordinary culture media, regards B. aerogenes capsulatus as capable, by itself, of exciting inflammatory purulent processes. It is certain that there are various strains of B. aerogenes capsulatus as regards pathogenicity and that the different results obtained by different investigators with respect to that feature are due to this fact. Cultures of B. aerogenes capsulatus made by Mr. Ward from the feces of a young man with slight digestive derangement and from material derived from a case of pernicious anæmia were injected into the breast muscles of pigeons (according to a suggestion from Dr. Flexner, who had found these animals to be especially susceptible). The cultures set up a localized necrotic inflammation with gas production in the connective tissues. Death occurred, apparently from toxæmia, within twenty-four hours. The application of these facts to capsulatus infection of the intestine is not now clear. It is certain that the pathogenicity of B. aerogenes capsulatus is different for different strains isolated from the human digestive tract. Thus, Professor Theobald Smith sent us an organism (anaerobe xxxi) which he isolated from the stool of a patient with pernicious anæmia and which proved to be much less pathogenic for guinea-pigs than the typical form of B. aerogenes caspulatus. This organism differed but slightly from the typical bacillus in its morphology, but showed the important peculiarity of being non-hæmolytic. It fermented the various sugars, but the gas production was less abundant than in the case of the typical gas bacillus. The gas production and decomposition induced in an incubated rabbit were also less pronounced than in the case of the typical B. aerogenes capsulatus.

Agglutination.—A single observation was made by Mr. Ward on the blood serum from a patient with pernicious anæmia (and capsulatus infection of the intestine) with respect to a possible

[1] "Zur Aetiologie der Gasphlegmone," *Centralbl. f. Bakt. etc.*, I Abt., Orig., xxxv, No. 6, pp., 554, 686, 1904.

agglutination action. The results were entirely negative.[1] It is interesting to note in this connection that Kamen[2] obtained no agglutinative action from the serum of rabbits which had been immunized with the gas bacillus. Positive results have, however, been recently obtained by Werner[3] who employed a special technical procedure in the immunization of the rabbits which served as experimental animals. The immune serum caused agglutination of the homologous gas-phlegmon bacilli (derived from a gas liver found in a fatal case of wound infection) in a dilution of 1:1000. Passini[4] obtained positive results in some of his cases not only with homologous strains but also with unrelated ones. In this case, however, the agglutinative action of the immune serum was less marked than in the case of the sera obtained by Werner. It is evident that there is still much to be learned in relation to the immunizing action of B. aerogenes capsulatus and that such an action in man may yet be discovered.

Summary of the Leading Characteristics of Micro-organisms of the B. Aerogenes Capsulatus Type Derived from Human Feces.

The following is a summary of the leading characteristics of the micro-organisms which have been referred to in this paper as belonging to the B. aerogenes capsulatus "type." By no means all of these characteristics have been established for the suspected capsulatus micro-organisms from each case of saccharo-butyric putrefaction which has been studied, but a sufficient number of characters have been determined to show that we have been dealing with bacteria either identical with those described by Welch under the name, B. aerogenes capsulatus, or so closely affiliated as to fully justify us in classifying them as examples of this micro-organism. The expression "capsulatus type" is used because there are sub-varieties of the micro-organism described by Professor Welch. The most important sub-variety

[1] Prof. Theobald Smith tells me that he obtained negative results in agglutinative tests made with the blood of a patient with pernicious anæmia who showed large numbers of B. aerogenes capsulatus in the feces.

[2] *Loc. cit.*

[3] "Die Agglutination bei Gasphlegmonbacillen," *Arch. f. Hyg.*, liii, p. 128, 1905.

[4] *Münch. med. Wochenschr*, li, p. 1283, 1904.

at present known to us is the one above described as being non-hæmolytic in action and less pathogenic for guinea-pigs than the common form of the organism. The capacity of the bacillus to hæmolyse or its failure to do so may prove to be especially significant in relation to the etiology of anæmia.

The organisms we have classed as belonging to the B. aerogenes capsulatus type are large, plump, usually straight bacilli, which, as they occur in the feces, can usually be shown to be provided with a capsule. Organisms which have developed in a living or dead rabbit always acquire capsules. The organisms occur very often in pairs, end to end, sometimes singly; sometimes in chains; sometimes as threads, which may be nearly straight or sharply bent on themselves. The ends of adjacent bacilli are slightly rounded or squared, though not so sharply squared as in the case of anthrax bacilli. They are immobile when viewed in hanging drops. Spore formation occurs with difficulty; *i.e.*, chiefly under very special conditions, such as on a medium containing blood serum or within the body of an animal. Occasionally spore formation is seen in blood-agar colonies, the bacilli from which in other respects conform to the characters of B. aerogenes capsulatus. On sugar-bouillon, gas formation is abundant and rapid, twice as much gas (or more) being formed in twenty-four hours as is usually formed by organisms of the B. coli group. The gas consists of from one-third to one-half carbon dioxide, the remaining gas consisting mainly of hydrogen.[1]

[1] Prof. Theobald Smith gives the following gas formula for B. aerogenes capsulatus: $\frac{H}{CO_2} = \frac{2}{1} = \frac{3}{2}$

The following table illustrates the approximate ratio of hydrogen and carbon dioxide:

Source of Micro-organism	Medium	Height of Gas Column in Millimeters (in 24 hours)	$\frac{H}{CO_2}$
1. Young man with slight digestive derangement	Milk	87	[illegible]
" "	Dextrose-bouillon-blood.	7	[illegible]

The gas production in incubated rabbits is very rapid and is associated with a characteristic sweetish, sickening odor of butyric acid mixed with some unknown constituent or constituents. The gas obtained from the peritoneal cavity and connective tissues gives the hydrogen "bark" and burns with a blue flame. The liquefaction of muscles, liver, etc., is remarkably rapid in such

Source of Micro-organism	Medium	Height of Gas Column in Millimeters (in 24 hours)	$\frac{H}{CO_2}$
2. Same case as No. 1	Milk	75	$\frac{2}{1}$
" " "	Dextrose-bouillon-blood	80	$\frac{2}{1}$
3. Pernicious anæmia (Case IX of tables)	Milk	60	$\frac{2}{1}$
" "	Dextrose-bouillon-blood	75	$\frac{2}{1}$
4. Pernicious anæmia (Case XII of tables)	Milk	70	$\frac{5}{3}$
5. Pernicious anæmia (Case VI of tables)	Milk	100	$\frac{2}{1}$
" "	"	85	$\frac{5}{3}$
6. Pernicious anæmia (Case VIII of tables)	Milk	90	$\frac{2}{1}$
7. Same case	Milk	84	$\frac{5}{3}$
8. Aerobic capsulate bacillus	Milk	87	$\frac{5}{3}$
9. Milk bacillus	Milk	90	$\frac{2}{1}$
10. Milk bacillus	Milk	85	$\frac{5}{3}$
" "	"	85	$\frac{5}{3}$

incubated rabbits. Grown on pasteurized milk, the bacteria in question induce rapid gas formation ("stormy fermentation") with disruption of curds into small masses. They do not quickly produce hydrogen sulphide or methyl mercaptan on ordinary sugar-free media but may perhaps make these sulphur compounds more readily on milk. Grown in fermentation tubes containing blood bouillon, they rapidly liberate hæmoglobin.[1] The organisms are strictly anaerobic and many of their colonies on blood agar appear after two or three days as minute points which lie beneath the surface and develop into fuzzy spheres. These spherical colonies often have dark centres. The micro-organisms induce inflammatory necrotic changes with gas formation when injected into susceptible animals, such as pigeons.

As first shown by Prof. Theobald Smith, B. aerogenes capsulatus usually grows readily on bouillon in the closed arm of the fermentation tube provided small bits of sterile, fresh tissue are introduced into it. The liver of the guinea-pig may advantageously be used. The presence of the tissue probably favors the growth in two ways: by furnishing a constituent of the medium necessary for the growth of the organism, and by inducing a more strict condition of anaerobiosis by the reducing activity of the cells.

On the Significance of Excessive Saccharo-butyric or Capsulatus Fermentation and Putrefaction in the Human Intestine.

Evidence has been advanced in the preceding pages to show that the regular presence of B. aerogenes capsulatus (or organisms of this type) in large numbers in the intestinal tract is a characteristic of certain cases of advanced apparently primary anæmias, whereas in ideal conditions of human digestion it is present in small numbers only or is not detectable by ordinary methods. It has further been shown that in the group of anæmias in question the representation of B. coli in the feces has in most instances been unsatisfactory. We are now in a position to discuss the biological meaning of this overgrowth of organisms of the capsulatus type and subordination of the B. coli group.

[1] As already noted in the preceding pages, there is a non-hæmolytic variety of B. aerogenes capsulatus.

If we cultivate B. aerogenes capsulatus on sugar bouillon we find that it is a large producer of gas (mainly hydrogen and carbon dioxide) and that it makes butyric and closely related acids in abundance, while the formation of lactic acid is small. On media which contain very little sugar but much proteid, the organism is still able to make gas in considerable amounts, though less freely than on a sugar medium, in which the liberation of gas is remarkably rapid. In nearly sugar-free media the gas bacillus produces butyric acid and the quantity of this in old cultures may be surprisingly great. Ammonia is formed at the same time and serves to neutralize at least in part the acid which is simultaneously made. The organism may apparently also produce a large quantity of indol on a suitable medium. Thus in a sterilized egg-meat medium after a growth of one month at 37° C., 100 c.c. of the filtered culture contained 16 milligrams of indol, but no skatol.[1] The egg and meat had undergone gradual solution and peptonization. Professor Theobald Smith tells me he regards the absence of indol production as characteristic of most strains of B. Welchii, although other strains produce it (for example one derived from a rabbit gave indol).

More important for the pathologist than any of these substances is the formation of a moderately hæmolytic substance or substances by the gas bacillus. Evidence of such substances was obtained in a five-day culture of capsulatus in blood bouillon. One-half of one cubic centimeter of the filtrate from this culture induced hæmolysis in a suspension of rabbit's red cells prepared by Ehrlich's method, the filtrate having been carefully neutralized to the litmus point. The same result was obtained in the case of red cells from a large Rhesus monkey. Treatment of this filtrate in an exhaustion apparatus very slightly reduced the hæmolytic action; heating to 70° C. for one hour reduced it still further; but even boiling did not wholly destroy it.

In order to determine whether this hæmolytic action was dependent in part on volatile ammonium compounds, the capsulatus filtrate was rendered distinctly alkaline with sodium carbonate and concentrated under reduced pressure at a low temperature for the removal of ammonia. The filtrate was then

[1]This culture contained besides B. aerogenes capsulatus small numbers of a diplococcus.

restored to its original volume by the addition of 0.85 per cent. salt solution. It was found that the hæmolytic action of the fluid was somewhat diminished but not lost.

The hæmolysing action of B. aerogenes capsulatus is very clearly shown in fermentation tubes containing sugar-blood bouillon which have been inoculated with pure cultures. A free liberation of hæmoglobin occurs in twenty-four hours or less. A similar result is seen in the case of an aerobic organism closely resembling B. aerogenes capsulatus in morphology. The bacillus of malignant œdema does not exert a hæmolysing action under similar conditions. B. putrificus was found to reduce hæmoglobin, but this change is much less marked in the case of B. aerogenes capsulatus.

There are other indications that B. aerogenes capsulatus makes a hæmolytic substance or substances. Rabbits injected with pure cultures of B. aerogenes capsulatus and then incubated at 37° C. soon show indications of hæmolysis, whereas control animals subjected to the same procedure do not exhibit an equal degree of hæmolytic change in the same period of time. This corresponds with the observation that advanced hæmolysis is usually noted in persons who at autopsy show signs of general invasion of the gas bacillus.

We have then in the gas bacillus an organism which gives rise to a distinctive type of decomposition, characterized by abundant gas formation, the production of butyric acid, and the formation of hæmolytic substances of partly unknown nature. On many media the organism produces a slightly sweetish, sickening odor, which is highly characteristic and which may be intense.

In persons whose intestinal contents hold the living gas bacillus in large numbers we find definite evidence of the saccharo-butyric type of fermentation, especially the production of gas in the colon (or higher up) and the formation of butyric acid. It has already been pointed out that butyric or allied volatile fatty acids are formed in abundance in some cases of pernicious anæmia in which flatulence is also common. This saccharo-butyric type of fermentation may probably persist, associated with notable flatulence, for many years without the development of a marked anæmia. When dependent on B. aerogenes

capsulatus, the flatus may have the peculiar sickening, sweetish odor which I have just mentioned or a garlic-like odor noted in certain cultures of capsulatus.

It is not yet clear whether the organism makes a substance capable of exciting an acute inflammation of the ileum or colon or whether preceding mechanical or chemical irritation is necessary to enable the organism to multiply rapidly and excite further inflammation. Healthy monkeys may be fed considerable numbers of capsulati without developing signs of inflammation in the intestine, although such feeding is followed by an increase in these organisms in the feces. Two monkeys fed on gas livers from incubated rabbits infused with pure cultures of B. aerogenes capsulatus developed soft stools temporarily. Such experiments are, however, quite different from the experiment of introducing capsulati into a digestive tract already somewhat inflamed and irritable in consequence of preceding infections. The ability of B. aerogenes capsulatus to cause an inflammatory necrotic process in the muscles of guinea-pigs and pigeons, which was noted by Dr. Flexner many years ago, is of interest in this relation. It appears probable that B. aerogenes capsulatus is often the cause of slight inflammatory or perhaps even necrotic changes in the mucous membrane of the intestine. Howard[1] has described instances of superficial necrosis of the mucous membrane of the stomach and intestine, associated with the presence of capsulatus in abundance These necrotic areas most often lie beneath the folds of the valvulæ conniventes and may occur with gas cysts. It does not seem likely that B. aerogenes capsulatus is responsible for severe acute inflammatory lesions of the intestine, but it is probable that its activities will account for the subacute enteritis that is so often present in cases that show large numbers of the bacilli in the stools. It is certain that there are many instances of acute diarrhœa associated with very free capsulatus multiplication and such diarrhœas are common in persons with severe primary anæmia. In one of our cases the disease set in soon after a period of intestinal diarrhœa contracted during the summer, and this

[1] *Contributions to the Science of Medicine, Dedicated by his Pupils to William Henry Welch on the 25th Anniversary of his Doctorate*, Baltimore, 1900, p. 461.

diarrhœa has been etiologically connected with the capsulatus infection.

The saccharo-butyric putrefactive process in the intestine, when active, is associated with an abundant liberation of gas, consisting largely of hydrogen. It therefore appears safe to attribute to B. aerogenes capsulatus much of the intestinal flatulence which is noted in persons suffering from digestive derangements characterized by the presence of capsulatus in great abundance. A breast-fed child (in the case of Dr. Holt), suffering from chronic capsulatus diarrhœa, was greatly troubled by flatulence until it received a milk containing less sugar. The ability of capsulatus to liberate hydrogen, even on media containing little carbohydrate material is one evidence of exceptional reducing power and I am disposed to connect this reducing power with an important though somewhat obscure characteristic of the feces in advancd anæmias.

It has already been noted that among our cases of anæmia there were few which did not give a strong mercuric chloride reaction in the feces, a reaction attributable to hydrobilirubin. As I have met with the strongest bichloride reactions in cases where capsulatus was very abundant in the feces, I am inclined to believe the reduction of bilirubin to hydrobilirubin is effected in these cases with the help of capsulatus. It has not yet been possible, however, to induce the reduction of bilirubin to hydrobilirubin experimentally by means of capsulatus activity, possibly because this reduction requires a symbiotic action not yet reproduced.[1]

The fecal extracts obtained from many persons with "primary" pernicious anæmia or with certain chronic intestinal derangements may exhibit distinct hæmolytic action toward the red cells of rabbits and monkeys, and in some cases this action is pronounced.[2] On the contrary the fecal extracts from healthy children on mixed diet may exhibit no appreciable hæmolytic action. The cause of the hæmolytic action just mentioned is

[1] Probably two factors are necessary to secure a highly excessive hydrobilirubin reaction: (a) excess of bilirubin in the bile, (b) excessive reducing action of bacteria in the intestine.

[2] In cases where the fecal extracts are deeply colored it is difficult to judge accurately of the hæmolytic action.

not yet clear. Indol may be present in considerable quantities in the intestine and its solutions exhibit a slight hæmolytic action for rabbits. Ammonium compounds are also frequently present in moderate amounts, and it is well known that such compounds have hæmolytic properties. In saccharo-butyric putrefaction ammonium butyrate may perhaps be formed in sufficient amount to cause some hæmolysis within the intestinal capillaries, and to contribute to the hæmolytic action of the fecal extracts. But it is also possible that, as already stated, B. aerogenes capsulatus forms some complex organic hæmolytic substance. Much more careful investigation of this point is necessary before drawing any conclusions as to its possible pathological significance.

The relation of capsulatus to the formation of indol in the intestine is not clear. Ordinarily the organism appears not to be an indol producer. In some of the anæmia cases, indol formation in the intestines has not been a marked feature although capsulatus was abundant. In other cases large amounts of indol have been formed. In such instances we may either have to do with a strain of capsulatus which is a good indol maker or with the symbiotic action of capsulatus and B. coli or some other intestinal micro-organism. I am inclined to believe that other bacteria than B. aerogenes capsulatus are generally concerned in putrefaction when indol formation is large in the gut.

We may consider now some of the conditions of the intestinal tract in which B. aerogenes capsulatus is present in excessive numbers and exerts pathological effects.

Even in early life the intestinal tract is not rarely the seat of capsulatus infection. Careless feeding apparently offers the conditions favorable for the temporary establishment of the organism, which manifests itself in irregularities of the bowels, diarrhœa, movements showing saccharo-butyric putrefaction, and ultimately anæmia. In Case XIV we have an example of capsulatus infection in a young child fed on cow's milk in which a severe form of anæmia was developed in the course of a few months. Tissier[1] has described an intestinal infection in breast-fed and bottle-fed children, which occurs especially in warm

[1] *Ann. de l'Inst. Pasteur*, xix, p. 273, 1905.

weather. The disease is attributed by him to the organism known to the French as B. perfringens. It appears, however, that B. perfringens is identical with B. aerogenes capsulatus. It appears highly probable that the diarrhœas of some breast-fed infants are to be referred to capsulatus infection.

From normal infancy and child-life to the time of adolescence the movements are remarkably free from B. aerogenes capsulatus. They can generally be isolated even from such material, but only in such small numbers as to be probably insignificant physiologically and pathologically.[1]

In this connection it should not be forgotten that Klein isolated from the feces in an outbreak of diarrhœa an organism which very probably is identical with B. aerogenes capsulatus. This organism was given the name B. enteritidis sporogenes. The position of the organism described by Klein is open to some suspicion because while it is stated by him to produce gas and butyric acid in the very characteristic manner observed by Welch and Nuttall for B. aerogenes capsulatus it differs from the latter in having motility and apparently in sporulating much more readily. Cultures sent by Dr. Klein to Professor Welch contained bacilli which agreed in every detail with pure cultures of B. aerogenes capsulatus.[2] As, however, the feces sometimes contain bacteria having the morphology of B. aerogenes capsulatus but differing from this organism in forming spores much more readily it is possible that Klein was in reality dealing with impure cultures of B. aerogenes capsulatus.

In early adult life B. aerogenes capsulatus is often present in the intestine in considerable numbers, even when health is apparently good. I have not, however, observed any instances in which it was very prominent among the fecal bacteria without some signs of intestinal disturbance, with evidence of excessive intestinal putrefaction or more obvious symptoms, such as diarrhœa and flatulence.[3]

[1] Hirshberg, working with Professor Welch, found the organisms to be widely distributed in the intestines of animals.

[2] See *Manual of Bacteriology* by Muir and Ritchie, American Edition, 1904, p. 354.

[3] In one instance which has come under observation the feces contained abundantly an organism appearing to be B. aerogenes capsulatus, although the subject was not ill in any ordinary sense. The subject was a woman

There are many persons who have from time to time slight diarrhœal disorder associated with a great temporary abundance of B. aerogenes capsulatus and streptococci in the stools, but who keep in fair health for years in spite of these recurrent seizures. These persons are apt to develop a moderate grade of anæmia and an intolerance for vegetable acids and carbohydrates. The intolerance for carbohydrates is probably connected with the presence of capsulatus in the small intestine. We know that this organism grows readily on carbohydrate media and it is likely that it multiplies, in many persons, in the small intestine, where carbohydrates have been freely eaten. The descent of the organism to the colon in large numbers is followed by active saccharobutyric putrefaction and its consequences. I have noticed capsulatus growing in long threads in the feces of a person who had diarrhœa following an excessive carbohydrate meal. This thread-like development may also be observed in the livers of dead rabbits which have been incubated after intravenous infusion of capsulatus. In both cases the thread-form is attributable to an abundance of carbohydrate pabulum.

It is highly probable that there are few persons who do not from time to time suffer intestinal derangements connected with over-multiplication of organisms of the capsulatus type and that normal bacterial conditions are in most cases quickly re-established, after the subsidence of these seizures. But there may also come a time in the life-history of an individual who has had these seizures repeatedly when the capsulatus organism fixes itself persistently in the intestinal tract and assumes a pseudo-parasitic instead of a saprophytic relation to the host. The capsulatus organism is then found to be abundantly and regularly present in the movements. Coincidently there is a decline

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eighty-five years of age in a remarkably good state of preservation. She was distinctly feeble and felt herself growing gradually feebler, but was only moderately anæmic. In this case there was an inclination to constipation and the urine and feces were uncommonly free from putrefactive products. It should be noted, however, that this comparatively good condition was maintained only by the utmost moderation in diet, the avoidance of sweets, highly seasoned food, etc. Slight indiscretions were regularly followed by diarrhœa and prostration. Micro-organisms of the B. coli type were abundant and well preserved in the fecal field, and gas production was moderate on the part of the fecal flora.

in the general health—often a very slow decline—characterized by loss of strength, slight anæmia and often, though not necessarily, loss in weight. Evidences of excessive putrefaction are commonly, though not invariably associated with this slow decline in physical status—a decline which at this period of the process may be temporarily or indefinitely checked by suitable measures. In more extreme instances the process of capsulatus decomposition within the intestine attains a high degree of intensity, partly on account of the large numbers of the organism, partly, perhaps, owing to the development (or original possession) of more than usually marked pathogenic properties on the part of the micro-organism, partly, perhaps, because a large tract of the intestine is implicated.[1] These extreme instances are marked by the development of a high grade of anæmia. Indications are not wanting that quantitative alterations in the blood are present, often for a long time before the hæmoglobin falls greatly or the red cells are much reduced in numbers. Ultimately there develops the blood picture of a severe secondary anæmia or the picture of pernicious anæmia.

In those cases where the feces contain regularly very large numbers of B. aerogenes capsulatus and few living organisms of the B. coli type, it is not yet clear which is the order of events in the establishment of this abnormal relationship—whether the elimination of B. coli tends to overgrowth of the gas bacillus or whether a great increase in the gas bacilli leads to the suppression of the colon bacillus. It seems probable that the dominance of the one type necessitates conditions which favor the restriction of the other. For the strictly anaerobic condition which is necessary to the growth of the gas bacillus is in itself inimical to the best development of B. coli. On the other hand B. coli thrives under fairly aerobic conditions which in themselves quite check the gas bacillus, although it is conceivable that under some circumstances B. coli may favor the growth of the gas bacillus by the appropriation of oxygen.

[1] Of the conditions of capsulatus activity in the small intestine we at present know nothing, but the suspicion seems not unwarrantable that the organism under some conditions multiplies actively even in the upper part of the small intestine. The chief evidence of this is the presence in the small intestine of micro-organisms morphologically resembling B. aerogenes capsulatus in some persons dying from intestinal disorders.

According to the view here put forward pernicious anæmias and secondary anæmias are often closely connected with a toxic-putrefactive process in the intestine initiated by a gas-forming organism of wide distribution—B. aerogenes capsulatus. The strongest arguments in favor of a causal relationship in some cases of anæmia are (1) the abundance of this organism in the feces of such patients as compared with their moderate occurrence in the feces of normal individuals; (2) the gradual reduction in the number of these organisms as the clinical conditions undergo improvement; and (3) the ability of the B. aerogenes capsulatus to make a hæmolytic substance or substances. Considerable work remains to be done to establish the nature of the hæmolytic action of the capsulatus organism and it also remains to be seen whether it is possible by means of capsulatus products to induce extreme forms of anæmia in animals closely related to man. In the absence of the latter evidence the dependence of anæmia on capsulatus infection is an hypothesis rather than an established fact, but it is one which is put forward as already possessing a high degree of probability. The fact that streptoccocus infection is sometimes associated prominently with capsulatus infection in advanced anæmias does not, I think, weaken the significance of the association of anæmia and capsulatus infection.[1] But it is to be distinctly emphasized that the claim here made is not that severe anæmia is an invariable or exclusive result of capsulatus infection of the intestine, but rather that it is an important incident of certain extreme cases of chronic capsulatus infections.

It is possible that an important factor in determining the nature of the pathological action exerted by B. aerogenes capsu-

[1] There certainly are instances of streptococcus infection of the intestine (sometimes of oral origin) in which there develops a moderate or pronounced anæmia which may reasonably be referred to the influence of this infection, since evidence is wanting of the presence of other pathogenic micro-organisms in significant numbers. There are also instances in which we have to deal with a combination of streptococcus and capsulatus infection. Among our cases of anæmia there are a number in which streptococci were regularly very abundant in the microscopical fields, and cultures from such cases have shown streptococcus to be dominant. The significance of combined streptococcus and capsulatus infection of the intestine is not yet clear. The ability of certain streptococci to produce

5. The infection of the intestinal tract with B. aerogenes capsulatus leads to varying clinical results according to the intensity of infection, its duration and its bacteriological associations. Moderate numbers of the bacilli administered to monkeys cause only slight diarrhœa with increase of the capsulatus organisms in the stools. There are frequent diarrhœal conditions in breast-fed and bottle-fed children which seem clearly referable to B. aerogenes capsulatus. Many temporary intestinal derangements in adults are associated with a temporary increase in B. aerogenes capsulatus. In children capsulatus infection may lead to the development of extreme anæmia with general œdema.

6. In many acute and subacute capsulatus infections of the intestine, the living micro-organisms of the B. coli type in the feces are much reduced in number. This condition of colon bacillus sparsity is shown (a) by the appearance of the Gram-stained fecal fields; (b) by the diminished production of gas in fermentation tubes; (c) by the failure of the sediments in fermentation tubes to show a satisfactory growth of organisms of the B. coli type on sugar bouillon; and (d) by the small number of colonies found on gelatin plates or their entire absence.

7. Chronic conditions are not rare in which the feces show a condition of colon bacillus scarcity associated with a marked increase in the numbers of B. aerogenes capsulatus. The persons in whom these bacterial conditions have been persistent almost always are at least moderately anæmic, *i. e.*, show some fall in hæmoglobin and indications of a diminished volume of blood.

8. B. aerogenes capsulatus is prominently characterized by the ability to induce a characteristic type of putrefactive decomposition which may be designated *saccharo-butyric putrefaction*. Among the chief products of saccharo-butyric putrefaction are carbon dioxide, hydrogen, butyric acid, and ammonia. This putrefaction may occur on proteid media containing very little carbohydrate material.

9. The excessive formation of gas and the consequent flatulence in many cases of capsulatus infection of the intestine are referable to excessive saccharo-butyric putrefaction or fermentation.

10. The excess of higher volatile fatty acids, including

butyric, which is observed in many cases of capsulatus infection is referable to excessive saccharo-butyric putrefaction.

11. B. aerogenes capsulatus is an active hæmolysing agent. The hæmolysis induced by it *in vitro* can probably be referred in part, but only in part, to the formation of ammonium butyrate.

12. Many instances of "primary" pernicious anæmia and of secondary anæmia show pronounced indications of excessive saccharo-butyric putrefaction, but the fecal and urinary manifestations of this process differ considerably in different instances. An increase of indol in the feces and of indican in the urine are common, though not regular manifestations. A high grade of intestinal putrefaction with excessive formation of phenol and indol may occur in persons who show few capsulati in the feces.

13. There are instances of "primary" pernicious anæmia and others showing the blood changes of secondary anæmia (of undeterminable etiology), in which there is chronic infection of the intestinal tract with B. aerogenes capsulatus. The gas bacillus is in these cases the dominant spore-forming anaerobe. The representation of living micro-organisms of the B. coli type in the feces is usually much reduced in these cases.

14. In certain instances of advanced anæmia it has been observed that as the blood picture and the general conditions improve there is a distinct reduction in the numbers of B. aerogenes capsulatus in the feces together with a better representation of the B. coli group. With these altered bacterial conditions there is usually an increase in the capacity for gas production by the mixed fecal flora.

15. The close association between certain anæmias and capsulatus infection of the gastro-enteric tract creates a presumption that this infection stands in a causative relation to these anæmias although experimental evidence of such a relation has not yet been obtained.

16. In some of the anæmias associated with capsulatus infection, large numbers of streptococci are found in the feces. The significance of this mixed infection is not yet clear.[1]

[1] The following practical considerations respecting infection through the agency of food deserve mention:

An important practical measure in all cases of capsulatus infection is the avoidance of food containing B. aerogenes capsulatus. As an organism

17. It sometimes happens that the numbers of B. aerogenes capsulatus in the feces undergo such a great decrease in the course of treatment that the microscopical fecal fields present a nearly normal appearance with respect to this organism. Therefore the failure to find B. aerogenes capsulatus in large excess after a patient has for some weeks or months had the benefit of treatment by diet and rest, does not necessarily exclude a preceding infection.

ABSTRACT OF CLINICAL NOTES TO CASES REFERRED TO IN THE FOREGOING PAPER.

CASE I. Presbyterian Hospital, service of Prof. James. Male, æt. about 45. Increasing weakness of legs before entry to hospital, some looseness of bowels, occasional vomiting. Hb. = 30 per cent.; red blood cells, 1,248,000; leucocytes, 5200; normoblasts, 1 per cent.; megaloblasts, 3 per cent.; many megalocytes; marked poikilocytosis. Later examinations with essentially same results.

CASE II. New York Hospital, service of Prof. Lambert. Woman æt. 58. Frequent diarrhœa with increasing weakness for past year with loss of 20 lbs. weight. Complains of weakness. Hb. = 35 per cent.; red blood cells, 1,440,000; red cells show marked granular degeneration. Many normoblasts, no typical megaloblasts, mitosis in normoblasts, Red cells larger than normal. No typical megalocytes. Later examinations with similar results.

CASE III. French Hospital, service of Dr. N. B. Potter. Woman æt. 65. Malaria 15 years ago in Smyrna, but well until 1 year ago. Since then, gradual loss of strength and color. Is now thin, toothless, and very pale. Hb. = 40 per cent.; red blood cells, 1,776,000; leucocytes, 5000; megaloblasts, 6, and normoblasts, 7 to 100 leucocytes. Patient shows little tendency to improvement as result of usual treatment for anæmia.

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identical with B. aerogenes capsulatus or very closely related to it (Grassberger and Schattenfroh's granulo-bacillus saccharo-butyricus immobilis liquefaciens) is often found in milk, it is especially important that attention should be given to the anaerobic micro-organisms of the milk and milk products employed as food. As sewage generally contains large numbers of B. aerogenes capsulatus, oysters exposed to sewage may become a source of intestinal disease from this micro-organism.

The official examination of milk by Health Boards might advantageously include the simpler tests (especially the action on milk resulting in "stormy fermentation") for the presence of saccharo-butyric putrefactive, spore-forming micro-organisms.

Case IV. St. Luke's Hospital, service of Dr. Spalding. First admitted 2 years ago with pneumonia and anæmia. Hb. = 35 per cent.; red blood cells, 1,328,000; megaloblasts found. No free hydrochloric acid. Since then has been very weak, with much emesis, anorexia, diarrhœal and sometimes bloody stools and cold extremities. Typical result of one of several recent examinations: Hb. = 41 per cent.; red blood cells, 1,032,000; leucocytes, 4000; polymorphonuclears, 14 per cent.; lymphocytes, 26 per cent.; normoblasts; aniso- and poikilocytosis.

Case V. Presbyterian Hospital, service of Dr. Thacher. Male, æt. 64. For 6 months dyspnœa and palpitation with loss in strength and weight and increasing pallor. Three weeks before admission, severe seizure of diarrhœa following retching after severe exertion. No teeth except two in lower jaw. Slight œdema over whole body. Slight rise in temperature. Repeated examinations of blood; typical one as follows: Hb. = 30 per cent.; red blood cells, 1,500,000; leucocytes, 3300; red cells show pallor; many myelocytes; marked poikilocytosis, polychromasia, and basophile granulation; normoblasts and megaloblasts present.

Case VI. New York Hospital, service of Dr. Lambert. Woman, æt. 49. Gradually increasing dyspnœa, palpitation, weakness, and headache. Nothing in previous history to give clue to etiological factor. Typical examination, Hb. = 33 per cent.; red blood cells, 976,000; leucocytes, 7400; many oval reds, moderate poikilocytosis; typical megalocytes. Normoblasts found in some examinations.

Case VII. Presbyterian Hospital, service of Dr. Thacher. Male, æt. 40. Small and thin. Pulmonary and testicular tuberculosis, evening temperature. Bowels irregular, movements soft. Hb. = 28 per cent.; red blood cells, 1,288,000; leucocytes, 9000; polymorphonuclears, 50 per cent.; transitionals, 7 per cent.; large mononuclears, 6 per cent.; lymphocytes, 27 per cent.; megalocytes and megaloblasts. On light diet, lavage of colon, and arsenic patient gradually improved. Three months after admission, Hb. = 62 per cent.; red blood cells, 3,792,000; leucocytes, 7900. Patient's color and strength steadily improving.

Note. This is one of the cases in which improvement coincided with a great diminution of B. aerogenes capsulatus in the feces and with a return of a good representation of bacteria of the B. coli type.

Case VIII. Roosevelt Hospital, service of Prof. James. Male, æt. 38. Typhoid fever 7 years ago; 2½ years ago, jaundice for one year. Since jaundice, weakness, dyspnœa, and anorexia. Sallow, fairly nourished, two carious molars. Hb. = 30 per cent.; red blood cells, 1,768,000; leucocytes, 6300; myelocytes, megaloblasts, normoblasts, poikilocytes, Other examinations with similar results.

Case IX. Roosevelt Hospital, service of Prof. James. Male, æt. 63. Weakness, anorexia, pallor, and dyspnœa for a year. Etiological factors

not discovered. Repeated examinations of blood of which following is typical: Hb. = 45 per cent.; red blood cells, 1,656,000; leucocytes, 13,000; megaloblasts, megalocytes, microcytes, macrocytes, polychromatophilia, poikilocytosis. Blaud's pills, Fowler's solution, colon irrigation, carnogen. Hb. fell to 30 per cent., red blood cells to 876,000. Death 2 months after admission.

CASE X. St. Luke's Hospital, service of Dr. Spalding. Male, æt. 30. Seven years ago became very weak and anæmic. Much digestive disturbance, alternating constipation and diarrhœa. Nausea, frequent vomiting of bile. Four years ago, Hb. = 25 per cent.; red blood cells, 928,000; two years ago, after temporary improvement, Hb. fell to 34 per cent.; red blood cells, 1,572,000; megaloblasts. No free hydrochloric acid. Temperature ranged from 101° to 102.5° F. On certain occasions megaloblasts abundant. After gradual improvement, was able to work 10 hours daily as switchman. One year ago entered hospital with all signs of pernicious anæmia. For a time patient's blood condition retrograded until Hb. was 25 per cent.; red blood cells, 660,000. After systematic colon irrigation, slow but steady improvement. After 3 months, Hb. = 90 per cent.; red blood cells, 3,086,000. Discharged greatly improved and able to walk several miles without fatigue.

Note. The feces of this patient were repeatedly examined for anaerobic micro-organisms. During the period of most pronounced anæmia and weakness, B. aerogenes capsulatus was very abundant in the feces, as was also indol. At the height of convalescence there was noted repeatedly a marked decline in these micro-organisms and in the putrefactive products, in the feces. Marked increase in numbers of colon bacilli in feces during period of improvement.

CASE XI. City Hospital, service of Dr. Ransom. Female about 50 years of age. In ward several months for weakness and anæmia. Digestive history not carefully recorded. Hb. = 20 per cent.; red blood cells about 1,000,000; leucocytes, 9000; no megaloblasts; no nucleated red cells; no morphological changes except marked deformity of red cells. At autopsy nothing was found to account for the profound anæmia.

CASE XII. Notes furnished by Dr. Fred. Shattuck of Boston. Male, æt. 73. Always robust until about 8 years ago. At that time a digestive disturbance which resulted in loss of strength. Apparently full recovery. Nearly one year ago a severe diarrhœal seizure lasting two weeks and causing confinement to bed. At this time, slightly anæmic. Eight months ago had become weak and had lost weight, but continued at business. Bowels irregular with diarrhœal tendency. Somewhat later was confined to bed. About 6 months ago, red blood cells numbered 1,800,000; megaloblasts and normoblasts observed. Gradual improvement set in 3 months ago. Hb. = 82 per cent.; red blood cells, 4,010,000; no blasts. Patient gained steadily.

Note. Six months ago feces contained B. aerogenes capsulatus in abundance (relatively to B. coli). During period of improvement numbers of B. aerogenes capsulatus showed distinct falling off; quite recently the number has again increased to a point far above the normal.

CASE XIII. Vanderbilt Clinic, service of Dr. Patterson. Male, æt. about 40. Gradual development of anæmia and weakness during past year. Blood examination gives indications of pernicious type of anæmia.

CASE XIV. Nursery and Child's Hospital, service of Dr. Lyon. Girl, æt. 3 years. Enteritis during summers of 1904 and 1905. Abdomen large, spleen reaches middle line; liver 2 inches below ribs in nipple line; lymph nodes in axilla and neck moderately enlarged. Typical blood examination showed Hb. = 25 per cent.; red blood cells, 1,250,000; slight lymphocytosis; nucleated red blood cells; normoblasts, megaloblasts, some mitotic figures. Child was on a general diet.

The diagnosis in this case was left in doubt. The bacterial and chemical examination of the feces and urine showed nothing which could be regarded as accounting for the observed anæmia.

CASE XV. Notes furnished by Dr. N. B. Potter. Male, æt. 45. For about 1 year, loss of weight, weakness, mental depression, and anæmia. Various derangements of intestinal digestion. No free hydrochloric acid Hb. = 35 per cent.; red blood cells, about 2,000,000. No improvement under influence of rest, iron, and arsenic. Diagnosis in doubt; carcinoma of stomach considered; also early stage of primary pernicious anæmia.

CASE XVI. Babies' Hospital, service of Dr. L. E. Holt. Female child, æt. 20 months. For several weeks, intestinal catarrh and diarrhœa Loss of weight, anæmia, very peevish, general œdema, casts in urine. By careful feeding, intestinal conditions were rapidly improved, œdema subsided, weight increased, and nervous symptoms subsided. Dec. 1, 1905, weight, 11 lbs., 13 oz.; Dec. 14, 13 lbs., 4 oz.

The blood history is as follows:

Dec. 1, 1905: Hb. = 35 per cent.; red blood cells, 1,798,400; leucocytes, 8000; polymorphonuclears, 38 per cent.; mononuclears, 58 per cent. large mononuclears, 3 per cent.; eosinophiles, 1; nucleated reds, about 2 per cent.; a good deal of basophilic degeneration in the reds.

Dec. 9, 1905: Hb. = 50 per cent.; red cells, 2,390,000; leucocytes, 15 per cent.; polymorphonuclears, 39 per cent.; small mononuclears, 50 per cent.; transitionals, 10 per cent.; some eosinophiles, numerous normoblasts, a few megaloblasts. Abnormal degeneration of red cells

Dec. 16, 1905: Hb. = 70 per cent.; red cells, 3,667,200; leucocytes. 12,000; polymorphonuclears, 51.6 per cent.; small mononuclears, 35 per cent.; large mononuclears, 8.25 per cent.; transitionals, 1.6 per cent.; eosinophiles, 1 per cent.; basophiles, 2.6 per cent.; nucleated reds, 1.

Dec. 23, 1905: Hb. = 75 per cent.

Note. At onset, the stools contained very large numbers of B. aerogenes capsulatus; B. coli were present in very small numbers. As improvement occurred, there was a return of B. coli and B. aerogenes capsulatus was greatly reduced in numbers.

Case XVII. Patient of Dr. Skinner, New Haven. Notes furnished by Prof. L. B. Mendel and Dr. L. F. Rettger. Male, æt. about 30. Formerly a painter. Is emaciated and appears wax-like. Hb. = about 20 per cent.; red blood cells, 380,000. Numerous nucleated red cells and megaloblasts. Treated by exposure to dry baking temperature. Rise to 2,600,000 red cells in short time.

The Collected Works of Carl Weigert.

E. K. DUNHAM, M.D., AND C. A. HERTER, M.D.
NEW YORK CITY.

Reprinted from The Journal of the American Medical Association, February 2, 1907, Vol. xlviii, pp. 417-418.

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ONE HUNDRED AND THREE DEARBORN AVENUE
1907.

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The Franco-Prussian War broke rudely into Weigert's studies, but his experience as surgeon to his regiment much widened his view of life. In later days he loved to tell of his military adventures during the seige of Paris and of his subsequent visit to England. During the years immediately following the war, Weigert gave considerable attention to clinical medicine in the Breslau Clinic and this education served him well in later life, when, with wonderful skill and insight, he indicated to the physicians of Frankfurt, in his talks in the dead-house, the relations between the pathologic findings and the clinical phenomena. It was during this early Breslau period that Weigert made his admirable investigations of the eruption in smallpox, in connection with which he developed the conception of cell death or coagulation necrosis and originated methods of staining bacteria in tissues. This research was rich in its yield of new methods, facts and ideas worked out by the young investigator.

But Weigert at this time made little impression on pathologic anatomists, not because his investigations were not original, but because, as Lichtheim says, they were too original. His work on smallpox, nevertheless, brought him to the notice of the great experimental pathologist, Julius Cohnheim, who soon took Weigert as his assistant at Breslau. The illness of the master threw a large part of the daily work of the institute and of the dead-house on Weigert, who soon developed under the critical eyes of his teacher into a pathologic anatomist possessed of superior technic and objective criticism. One might have supposed that Cohnheim's genius would have led Weigert into the growing and promising field of experimental pathology. There are two reasons why this proved not to be the case. Weigert early attained an individual view of medicine and of the methods most likely to advance it. He believed that pathologic histology might be materially advanced by the development of improved methods of staining, and from the outset was so successful in devising valuable histologic methods that he did not care to turn aside to join Cohnheim in his experimental studies. That Weigert's great master helped him by encouragement and suggestion is certain, but it is equally true that the young pathologist worked out his own salvation in all that pertained to histology. His independence extended still further and was early reflected in his well considered views of general biologic laws. That Cohnheim was influenced by the original thought of his pupil can not be doubted. For example, it appears to have been Weigert's influence that led the master, after having met with failure, to repeat with success his experiment on the inoculation of tubercles. But the strong individuality of Weigert as an original worker was not the sole obstacle to following the paths opened by the great experimentalist. A certain innate tenderness of nature, quite unmixed with

false sentiment, gave him an aversion to experimenting on animals—an aversion similar to that which made it impossible for Pasteur to do vivisection. Rieder tells us that the rabbits in the Senkenberg Institute led an enviable existence, for when they came in contact with their master it was to share his mid-day meal.

When Cohnheim, in 1878, was invited to fill the chair of pathologic anatomy at the University of Leipsig, Weigert went with him as extraordinarius, a position which he filled until the death of the master, in 1885. It was during this period that Weigert made some of his best contributions to pathologic anatomy; and perhaps no work of his has had a wider influence on the progress of medicine than the discovery in 1882 of a method by which the medullary sheaths of nerves can be sharply differentiated from the axis cylinder. On Cohnheim's death, Weigert undertook the duties incidental to teaching pathologic anatomy at the Senkenberg Institute in Frankfurt. Here, in the course of nearly twenty years' tenure of the directorship of the institute, he quietly continued his admirable researches in pathologic anatomy and placed many young investigators under an enduring sense of gratitude for the inspiration gained from his guidance.

Although the Frankfurt period was one of happiness for Weigert he never wholly recovered from the disappointment of failing to succeed Cohnheim in the professorship at the University of Leipsig, and throughout his life the failure caused him hours of depression. Weigert knew, what was equally well known to the best qualified judges, that he was the person best fitted to fill the chair vacated by Cohnheim, despite the fact that he was not an experimental pathologist. The thought that his colleagues had rejected him, very largely for the reason of his being a Jew, was a standing offense to his sense of justice. That his great merits were really in a measure recognized by members of the Leipsig faculty seems probable. They offered him a public call to the university on condition that he would not accept it—a proposition which naturally proved highly distasteful to Weigert. It is an indication of the superior nature of the man that in a manuscript dealing especially with the methods of making appointments in universities, found after Weigert's death, this difficult subject is dealt with in a thoroughly objective and judicial way, without the slightest intrusion of personal feeling.

Weigert resembled his great predecessor in pathologic anatomy, Virchow, in not being a good teacher for the man of average ability and poor training. He had none of the didactic ways of the schoolmaster and could not talk for artistic effect. It was thus fortunate that in Frankfurt he was not expected to give instructions to beginners in medicine.

The special students who came to his little laboratory prepared to do serious work found that Weigert possessed, in the highest degree, the power of intellectually stimulating others and of making their work fruitful. His influence as a teacher extended far beyond the field of medicine and deeply affected the philosophic outlook of many a student. The persons who came into close contact with Weigert recognized that the society of the joyous yet earnest man was ever an education and a delight.

As an investigator, Weigert belongs in a select group of deliberate, careful workers, who regard a problem calmly and from every side while bringing to bear extraordinary powers of analysis and a high degree of ingenuity in overcoming technical difficulties. The manner in which he developed his intricate methods of staining shows his capacity for grasping principles and applying them to special ends. His studies of inflammation and of new growths reveal the constructive philosophic mind, which derives its highest satisfaction in the search for fundamental laws in the midst of a bewildering maze of facts. Weigert's mind was one that advanced step by step and took few risks. His mind was almost too well ordered to lead him into the experimental ventures that produce the most strikingly original results. He never published until he was satisfied that he had done the best work of which he was capable, and when he said to one of his pupils in 1887, "One can never publish anything late enough," he gave the clue to his attitude toward research. In all that he did, Weigert had in mind soundness and conscientious performance. The extreme of this tendency sometimes had the detrimental effect on his work that may come from an exaggerated conscientiousness. For example, he was so anxious to perfect the reliability of his neuroglia stain that he let many more important subjects rest while he pursued an end which he could hope to attain only by a large admixture of good fortune with intelligent effort. Thus he tended at times to grow unproductive. In the admirable critical reviews which Weigert wrote, one finds the same conscientious performance as in his research work and the same interest in the perception of fundamental principles. The reviews on chemotaxis, on new theories of heredity and on antitoxin immunity, are cases in point and all bear the imprint of an original mind.

Weigert was a man of medium stature, with a large, well-shaped head. In his later years he was slightly inclined toward corpulence. His large brown eyes were beautiful, expressing gentleness and great intelligence. His sympathetic, open and joyous nature, which gave him interest in all kinds of human endeavor and all sorts of people, made him greatly liked. He mixed freely with scientific and practical men of all kinds. He often amused his more intimate friends with his powers of ventriloquism and mind-reading, but his greatest

social gift lay in a rare talent for telling stories full of a naive humor and kindliness.

Up to the time of his sudden death, from coronary thrombosis, he enjoyed good health and remained steadfastly at work. As his body lay on its bier there stood filtering in the laboratory a solution designed to improve the neuroglia stain. In the last years of his life Weigert devoted much thought to the pathogenesis of new growths and it was his intention to embody his views in a publication dealing with the subject from the standpoint of the laws of cell development. He was deeply interested in De Vries' great work on Mutation and believed it shed important light on some questions connected with the aberrant growth of animal cells. One of the reviewers visited Weigert in his laboratory not long before his death and found him sitting on a high stool with legs folded under him tailor-fashion, perusing De Vries'. "I read this work," he said, "over and over again. Parts of it I find very difficult to understand because it is so technical, but I do not wish to lose a line of it or miss an idea, and so I stick to it."

Despite his many and great contributions to medical science, Weigert in his later days had periods of depression in which he suffered great discouragement in regard to his capacity for work. He felt his powers waning and imagined his researches to be unimportant. On one occasion, when asked about his work he said, "I am working away at the old things—small, insignificant things. I realize that I can not compete with my younger colleagues. Look at my cousin Paulus (meaning Ehrlich) and his immunity work. How can I keep up with that? Then again this modern chemical pathology which requires so much special training. It is all right for Paulus with his extraordinary memory for those hexagons (benzene ring derivatives), but I can not do it."

The attitude of the universities toward Weigert doubtless contributed to his despondency and even made him at times doubt somewhat his powers, his knowledge and his worth. During the period of nearly twenty years following Cohnheim's death he did not once receive a university call—a fact difficult to understand when one realizes how greatly his figure towers above nearly all contemporary pathologic anatomists. He sought neither fame nor honors, but it was hard to be slighted for men of clearly inferior capacity. It is probably true that the scant appreciation shown him by the faculties was in part owing to lack of forcefulness and will power in certain directions. Rieder aptly says of him: "He had no idea how one makes a career and how necessary it is to-day to associate one's own advantage with that of others." Weigert clearly had the amiable faults of the over-sensitive idealistic student whose modesty makes it impossible for him to appraise his own worth.

In 1904 Weigert was looking forward with eagerness to a visit to the United States, to "the land of unlimited possibilities," as he liked to call it. It was expected that he would lecture at the Johns Hopkins Medical School on certain topics in general pathology on which he had long pondered. It is certain that he would have been enthusiastically received by his many friends and pupils and the visit would surely have helped him to throw off his doubts as to his own merit. "It will bring fresh wind to his sails," said his cousin, Ehrlich, in speaking of the proposed visit. But Fate willed it otherwise. In the summer of 1904, at the close of a Sunday agreeably spent with his friends, Weigert retired to his room to read, as was his wont. The next morning his lifeless body was found. He had apparently had no premonitions of what was impending. Indeed, during the last days of his life his companions had observed with pleasure a return of the buoyancy of spirit that had characterized his earlier days.

The death of Carl Weigert was in every sense premature. Through it humanity lost a singularly simple, noble spirit and the science of medicine was robbed of one of its greatest lights.

WEIGERT'S WORKS.

In these two handsome volumes, Weigert's works fill 1,328 pages, which are preceded by a preface and 140 pages devoted to a sketch of the master's life, tributes to his services in neurology and histology by Edinger and Ehrlich, and a chronologic list of Weigert's scientific publications in which reference is made to 97 titles.

It would be a hopeless task to attempt a detailed review of this rich collection. It is possible merely to select certain of these works for particular mention and this may be done almost at random, where all the material bears the marks of deep study and careful exposition. The style is exceedingly lucid and on the whole simple, but so concise and idiomatic as to render translation into exactly equivalent English a matter of great difficulty.

Weigert's works are grouped under six heads: "Bioplastik," "Pathologic Anatomy," "Pathologic Histology," "Bacteriology," "Neurology and Microtechnic," and "Varia;" the last including an obituary of Julius Cohnheim, his master, an article on mind-reading and some remarks referring to the establishment of an institute for experimental therapy in Frankfurt. This division does not follow the chronological order, but brings together those contributions which are naturally related to each other. In some respects the articles included under the collective title "Bioplastik" are of particular interest because they serve admirably to illustrate the breadth of thought manifested by Weigert throughout his writings and his analytical, critical and constructive abilities.

A striking example of the philosophical inclination of Weigert's mind to unify, systematize and correlate the knowledge he possessed is contained in the 186 pages bearing the caption "Attempt at a general pathologic morphology based on the normal," which is the seventh and final division of that part of the whole work designated as "Bioplastik." This study comprises fifteen chapters, only five of which may be regarded as completed, in at least a tentative form, to the satisfaction of the author, for in an interesting foot note of the editor's there is mention of Weigert's characteristic habit of repeatedly revising his writings. An enumeration of these chapters is all that is permitted by our space, but it will suffice to indicate the interest and value of this contribution, as well as the methodical way in which Weigert developed his ideas:

1. An Introduction and Plan.
2. Causality of Vital Phenomena.
3. General Considerations Concerning Function and Matter.
4. Origin of Living Matter.
5. Evolution and Epigenesis.
6. Idioplasm.
7. The Non-idioplastic Germ-Constituents.
8. The Multiplicity of the Germ-Potentialities in Phylogeny and Ontogeny.
9. The Alleged Totipotentiality of the Idioplasm of Somatic Cells.
10. Remarks on the Nature of the Changes in the Idioplasm During Ontogenesis.
11. External Conditions Activating Latent Idioplastic Rudiments.
12. Bioplastic Phenomena. Kinetic and Potential Bioplastic Energy.
13. Regeneration.
14. The Obstacles to Growth Concerned in Regeneration.
15. Idioplastic Activities in Regeneration.

We have selected this study from among those classed as bioplastic, for more detailed notice, partly because it is the final word from this liberal thinker upon these subjects, partly because it is a posthumous work, not published elsewhere. The other works placed in this category by the editors, with the dates of publication, are:

1. Inflammation, 1880 and 1886.
2. Vital Phenomena of Cells Under Pathlogic Conditions, 1886.
3. New Problems in Pathologic Anatomy, 1896.
4. New Theories of Heredity, 1887.
5. Recent Works on the Theory of Antitoxic Immunity, 1898.
6. Chats on the Methods of Research in Natural Science, 1898 (first published after Weigert's death).

At the risk of exceeding the limits proper to a review, we can not forbear making brief mention of the work on inflammation. The origin and foundations of our conception of this varied process are subjected to a critical examination based on an historical study of the modifications that conception has passed through, as insight into biologic processes has developed. This constructively critical survey of past achievements

leaves the wholesome impression on the reader's mind that knowledge is still in process of evolution and that the final word is not yet uttered. Having, nevertheless, classified and defined in this manner the known factors constituting our conception of the inflammatory process, the author analyzes and classifies its various manifestations in a characteristic and exceedingly lucid exposition of different concrete examples. No one can read such a broad and logical treatment of a complex subject without a refreshing sense of renewed inspiration.

The pathology of tuberculosis is discussed in thirteen articles, the first appearing in 1877, before the demonstration of the tubercle bacillus, and the last published in 1903. These studies, therefore, embrace the most interesting epoch in the development of knowledge concerning this disease and even in those articles which now have chiefly an historical interest one can not but admire the acute and thorough observation and the close and suggestive deductions of the writer. There are also several valuable papers on tumors, malformations, etc.

One of the subjects to which Weigert devoted much productive study is that of coagulation, both in the blood and tissues, and in these volumes will be found his successive papers elaborating the conception of coagulation necrosis and the technic with which the presence of fibrin and similar substances may be demonstrated by a differential stain. These articles afford another example of his patient effort to discover and define the essential facts and processes underlying biologic phenomena, and to make useful, conservative and guarded generalizations. One would be tempted, in this connection, to dwell upon Weigert's trained powers of imagination which led him to seek consistent hypotheses as an aid to research, were it not that the mere enumeration already made of the titles of the "Bioplastic" papers reveal this quality of his mind; a quality admirably blended with a critical judgment.

That Weigert was an expert histologist, hardly calls for mention. His services to that branch of medical science are well known. The studies of tissue-changes in coagulation necrosis rested upon this technical knowledge, but his widest influence in this direction was exerted by his elaboration of methods of staining with a view to identifying the various constituents of objects subjected to microscopic examination. His writings on these topics are embraced in the 345 pages included in the fourth and fifth divisions of this collection: "Bacteriology" and "Neurology and Microtechnic." The bacteriologic papers are not numerous and do not occupy more than 70 pages of a volume containing 774 pages. The first bacteriologic paper is one published in 1871 on the bacteria in the skin in smallpox. This paper is of interest as mark-

ing the first discovery of bacteria in tissues. Weigert shows his excellent judgment in this instance in not falling into the error of concluding that smallpox is due to the micrococci which he discovered in the lesions of this disease. The following paper is one on a mycosis in a newly born child and has to do with the coloration of bacteria. In 1881 Weigert published an important paper on the technic of microscopic investigations of bacteria in which he devotes special attention to methods of staining. Considerable space is devoted to methods of investigation of bacteria in sections. This communication also contains a section on the significance of the dyeing of bacteria. The author brings out here the importance of drying tissues with strong acetic acid or potassium hydroxid in order to render the bacteria capable of taking stáins in those cases where they have failed to be readily colored. It is interesting to note that although Weigert considers the introduction of the anilin dyes as extremely important in detecting the presence of bacteria in tissues, he does not draw the conclusion that the failure to take up color necessarily means the absence of micro-organisms. In other words, he clearly recogni es that further experiments are likely to result in the discovery of methods which will render visible micro-organisms which remain untouched even by the greatly improved methods developed by himself.

In 1887 Weigert published a controversial paper of considerable interest dealing with the bacteria question. Although the doctrines for which he contests have long since been established, it is interesting to read this paper even at the present day as an example of searching criticism of the contentions of a writer named Hiller, who energetically contended that his negative results with the inoculation of certain bacteria constituted a proof that these and most other bacteria are innocuous. In the same year Weigert published a paper on glycerin as a method of distinguishing formed and unformed ferments. The bacteriologic section is completed by three papers relating to Obermeyer's spirillæ of recurrent fever.

The section dealing with neurology and microtechnic constitutes one of the most important portions of the volume, embodying, as it does, Weigert's extremely important and fundamental methods for the differentiation of tissues. This section contains the following papers: First, a paper on microscopic technic which deals with the subject of section cutting, certain improvements in the microtome introduced by Weigert and the coloration and impregnation of preparations. This paper was published in 1894. It is followed by one dealing with the histologic technic of the central nervous system, published in 1896. This constitutes an admirable historical review of the subject and contains much of interest to histologists of the present day. It discusses the method of Golgi at great length and in a critical manner. The second contribu-

tion to the histologic technic of the central nervous system is dated 1897 and deals in an exhaustive manner with the subject of staining the medullary sheath of a nerve fiber and with the principles concerned in such staining. The third of this series of contributions on the histologic technic of the central nervous system is likewise dated 1897 and is devoted to the Marchi method. Then follow two papers dealing with the methods of staining fibrin. The first is dated 1887, giving Weigert's original method of selective coloration by means of anilin dyes. The difference between this method and that of Gram is very clearly brought out. This subject is brought up to date in a paper dated 1903, dealing again with the fibrin stain. These papers are followed by one dated 1898 giving Weigert's method of staining elastic fibers.

A section now follows which is devoted mainly to the papers of Weigert in which are presented his discoveries of methods of staining the medullary sheaths of the central nervous system. In 1890 Weigert brought out his extremely important method of staining the neuroglia structures of the central nervous system in man. His two papers dealing with this discovery are printed in this volume and are followed by a long contribution, of the utmost significance to histology, in which the normal human neuroglia is discussed from the historical standpoint and in the light of his own methods. This paper is dated 1895. Weigert's last contribution to the technic of the neuroglia stain was made in 1903, in which he treats of ways of improving his own, previously described, methods and also discusses similar methods for the study of neuroglia which have been developed by others. The rest of the volume is given up to papers of less importance. It should be mentioned, however, that among the papers which have been collected at the end of the volume is an extremely appreciative necrolog relating to Weigert's master, Julius Cohnheim. This paper was written in 1884. In the same year also was published a short discussion on the subject of mind-reading. The last contribution deals with the question of the establishment of an institute for serum investigation and experimental therapy in Frankfurt a-M., and will be read with interest by those who have followed the classical contributions that have come from this institute under the guidance of Ehrlich in the past nine years.

It was as a morphologist and resourceful microscopic technician that Weigert entered and left his impress on the field of bacteriology, and his services to neurology are of similar character. In fact, throughout his writings we find that the chief data underlying his work were morphologic. But he was pre-eminent in his ability to handle and verify these data. They inspired him to seek the significance of the changes in structure he was so capable of detecting, and this striving led

him to his conception of vital phenomena. The chemical and physical aspects of pathology are but lightly touched on, and when considered at all are discussed mainly in reviews of the work of others. That Weigert was in sympathy with these more recent aspects of pathology can not be doubted, but he was too completely engrossed in the problem on which he was best fitted to work, to make notable contributions in these directions. His publications in the field of his choice are destined to become classics.

The Common Bacterial Infections of the Digestive Tract and the Intoxications Arising Therefrom.

CHRISTIAN A. HERTER, M.D.
NEW YORK CITY.

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1907.

THE COMMON BACTERIAL INFECTIONS OF THE DIGESTIVE TRACT AND THE INTOXICATIONS ARISING THEREFROM.*

CHRISTIAN A. HERTER, M.D.
NEW YORK CITY.

After presenting some general considerations relative to the bacterial flora of the human digestive tract in health, and showing that none of the experimental studies made by investigators is really conclusive as to the necessity of bacterial action in the digestive tract for the maintenance of health in adult mammals of the highest type, Dr. Herter proceeded:

Clearly, then, the intestinal bacteria are not required to carry on the ordinary digestive processes of normal nutrition. It has been supposed that the intestinal bacteria aid in the digestion of cellulose which they are undoubtedly able to decompose fermentatively. This argument loses much of its force if it be true, as lately maintained by Bergmann, that most of the cellulose eaten by herbivora is provided with intracellular enzymes capable of decomposing cellulose.

The real significance of the normal intestinal flora probably lies, not in any immediate relation to processes of digestion but in a wholly different direction. It is impossible to avoid the entrance of bacteria into the digestive tract. The obligate bacteria (for example, *B. lactis aerogenes, B. coli, B. bifidus*) adapt themselves to the secretions of this part of the body and ordinarily hold their own against new-comers. By virtue of their adaptation, they are not ordinarily harmful to their host, but, on the contrary, they are, under some circumstances, capable of doing service by giving rise to condi-

* Abstract of the Harvey Society Lecture, delivered at the New York Academy of Medicine, Nov. 3, 1906.

tions that discourage the growth of many harmless and harmful species which man can not readily exclude from his digestive tract. I believe that the chief significance of the obligate intestinal bacteria lies in their potential capacity for thus checking the development of other types of organisms capable of doing injury.

Speaking of the defensive action of the digestive juices, Dr. Herter said that the normal human organism is provided with more or less efficient methods of defense against bacterial invaders. The acidity of the gastric juice, for instance, checks the growth of many nonsporulating bacteria and is, in a measure, destructive to most varieties. If, however, bacteria are administered in very large numbers, there is a chance that some of them will find their way into the intestine. This is particularly true when microbes are taken into the empty stomach or into a stomach with defective motility which secretes little gastric juice of low acidity. Dr. Herter goes on to say:

A long, largely anaërobic intestinal tract permitting gradual resorption of the contents is a physiologic necessity in order that a loss of water and its detrimental consequences may be spared the organism. The presence in the colon of immense numbers of obligate microorganisms of the *B. coli* type may be an important defense of the organism in the sense that they hinder the development of that putrefactive decomposition which, if prolonged, is so injurious to the organism as a whole. This adaptation is the most rational explanation of the meaning of the myriads of colon bacilli that inhabit the large intestine. This view is not inconsistent with the conception that under some conditions the colon bacilli multiply to such an extent as to prove harmful through the part they play in promoting fermentation and putrefaction. An alkaline reaction of the medium appears to favor their putrefactive functions if peptones be present.

The influence of reaction on the growth of intestinal anaërobes was studied very carefully by Dr. A. J. Wakeman, who found that the growth of putrefactive anaërobes is favored by neutral reaction and restrained by the presence of acids. This explains the favorable influ-

ence of milk (containing lactic acid formers) in controlling putrefactive decomposition in the digestive tract. Dr. Herter has this to say with reference to the aërobic and anaërobic conditions in the digestive tract:

AEROBIC AND ANAEROBIC CONDITIONS IN THE DIGESTIVE TRACT.

There are many conditions which influence the character and extent of bacterial decomposition in the alimentary tract: among them are the chemical character of the food, the solubility of the food in the digestive juices, and the volume and composition of these digestive juices. Intimately intermingled with these factors of food and secretory activity is the influence of aërobic and anaërobic conditions in the digestive tract on the nature of the bacterial activities that occur there. The initiation of putrefactive decomposition in the digestive tract, as elsewhere, depends very largely, though probably not exclusively, on the activities of obligate anaërobes. An important portion of the digestive tract is most of the time under anaërobic conditions.

The facts all point to the correctness of the view that we largely owe the initiation of bacterial proteid cleavage there to the agency of the strict anaërobes, but it does not follow that intestinal putrefaction is carried on through the sole activity of these organisms. The intestine abounds with micro-organisms, which are able to attack albumoses and peptones and to effect the further degradation of the proteid molecule, thus entering into a symbiotic action with the strict anaërobes.

The symbiosis of aërobes and anaërobes is a biologic phenomenon of much consequence in determining the distribution of anaërobic bacterial processes in the digestive tract. Without such symbiotic action, the development of strict anaërobes would be confined to those parts of the digestive tract into which oxygen passes rarely, and then only in small amounts. The large intestine is seldom visited by free oxygen, but it is probably usual in man for the small intestine to contain a little air.

It is probably safe to assume that in the mouth the free presence of oxygen constantly acts as a deterrent to anaërobic growth. In spite of this, however, anaërobic life is possible. Caries of the teeth, which was formerly

referred to aërobic bacteria, seems clearly the result of the invasive action of anaërobes on the tooth pulp. In removing decomposing food masses by the intelligent use of a tooth brush, one not merely admits air to the anaërobes, but also removes many aërobes, which, through the symbiotic action already mentioned, facilitate the multiplication of the former.

In a stomach which secretes little or no hydrochloric acid and which is sluggish in emptying its contents, the chances for anaërobic development are good, and hence we frequently find under these circumstances that there are evidences of putrefactive decomposition of food that has been unduly retained in the stomach (e. g., production of sulphuretted hydrogen, mercaptan, butyric acid, etc.). On the whole, however, I think one may say that in the course of chronic gastric affections the number of anaërobic micro-organisms in the stomach is seldom great.

Of the conditions of bacterial life in the small intestine, very little is known because of the inaccessibility of the contents of this portion of the digestive tract. However, observations at operation after gunshot wounds and at early autopsies have shown that putrefactive micro-organisms are commonly few in the upper two-thirds of the small intestines. In man there is in the ileum within a foot or two of the colon a marked increase, both in the number of bacteria and of their varieties. Hence we find that the mixed fecal bacteria taken from this level of the lower ileum are capable of inducing putrefactive changes in native proteids and in more simple nitrogen-holding media, even in health, and that anaërobic conditions of bacterial life are exaggerated in pathologic states. We may indeed look on the ileum as the debatable land of digestive territory.

In the large intestine we find the most dense accumulation of bacteria and the best conditions for anaërobic growth. The transition from small to large intestine is in this respect very striking. The anaërobic conditions are well maintained throughout the colon and it is here that we find the greatest numbers of anaërobes and the most pronounced evidence of putrefaction. There is, however, a gradual fall in the number of living bacteria beyond the ileocecal valve, so that in the rectum the numbers of cultivable bacteria are very much less than in the ascending colon. It should be noted, however,

that the variety of bacteria in this region is often not so great as in the ileum, although their numbers are in excess.

Dr. Herter then discussed the characters of the bacterial flora of carnivorous and of herbivorous animals and the reducing action of meat. He says that in the case of carnivorous animals living on raw meat there seems little doubt that anaërobic conditions may exist throughout the digestive tract, and that the reducing action of meat in the upper part of the tract may contribute materially to diminish the quantity of oxygen carried into the intestines. Meat which has been cooked slightly still possesses considerable reducing power, and it is not unlikely that there are cases of excessive intestinal putrefaction in man which depend on the excessive activity of anaërobes in which the conditions of anaërobiosis are favored by excessive meat eating.

Attention was also directed to the influence of the epithelial cells lining the digestive tract. Dr. Herter thinks that in cases where there is excessive production and absorption of indol (and of other noxious substances) this epithelium acts as a protective agency to the organism as a whole. Furthermore, the epithelial cells prevent the passage of bacteria from the lumen of the gut into the body tissue.

Evidence is gradually accumulating to show that pathogenic micro-organisms may be present in moderate or even in considerable numbers in the intestinal tract under some conditions without giving rise to clinical manifestations of deranged function. To quote Dr. Herter:

It is likely that in all these cases the pathogenic organisms in question are held in check by other bacteria present in the digestive tract or by the bacteria and the intestinal secretions, so that they are unable to multiply in a significant manner or to gain entry into the cells of the mucous membranes. It seems not unreasonable to suppose that this restraint may be overcome by errors in diet, depressed general conditions, or by alterations in the secretions of the digestive tract, and that thus definite infection by the hemiparasitic bacteria that are

present becomes possible. The considerations just mentioned as applying to these bacteria probably hold equally true of the more saprophytic forms concerned in intestinal putrefaction.

A variety of conditions may be presumed so to favor the development of these anaërobes that their products, instead of being formed in such small amounts as to be harmless, begin to exert a detrimental effect on the organism. Especially important are influences which alter the character of the secretions in the large intestines or bring there unusually large quantities of partly digested proteid food. In certain conditions of the digestive tract an excessive or even a moderate meal of proteid food will precipitate an intoxication or a seizure of vomiting or diarrhea. There are cases classed as ptomain poisoning in which the digestive tract rather than the food is responsible for the observed disorders.

It is evident that, while at all periods of life the human digestive tract contains numerous micro-organisms, the biologic characters of these organisms are not the same at all ages. In this may be found a cause for the different types and decomposition in the digestive tract. For instance, in the digestive tract of a nursing infant there is found a relatively simple bacterial flora, which should be a matter of interest to those who wish to obtain an insight into the physiology of digestion. The great majority of the bacteria are Gram-positive. Among these may be mentioned *B. bifidus*, *B. acidophilus*, *B. aerogenes capsulatus*, *B. lactis aerogenes*, and *B. putrificus*. As the result of his study of the distribution of bacteria in the intestine of the nursling from autopsies on babies dying in the first six months of life from causes not closely connected with the digestive tract, Dr. Herter presents the following summary:

In the normal nursling the mouth contains few bacteria and these are for the most part derived from the skin and the nipple—*Staphylococcus pyogenes aureus*, bacilli of the *B. coli* group and *B. lactis aerogenes*. In the stomach also the bacteria are few and the bacterioscopic picture shows usually a few positive or negative diplococci or streptococci, or negative coccobacilli, or positive or negative bacilli suggesting the *B. coli* and *B.*

lactis aerogenes groups. The normal bacteria of the greater portion of the small intestine are short Gram-negative bacilli of the colon and *lactis aerogenes* groups, mixed sometimes with a few positive and negative coccal forms. In the lower ileum the organisms of the bifidus type appear and at the transition from lower ileum to cecum there is a striking change in the proportions of coli and bifidus types, and the former lose their dominant numerical position. The ascendency of the bifidus type increases in the colon to such an extent that in the rectum this type has the appearance of being present in pure culture.

The bacterial flora of the intestinal tract of the nursling is thus only moderately numerous as regards variety. The bacteria are concentrated in the regions that lie between the lower ileum and the anus, the ileocecal junction presenting most organisms capable of being cultivated and the greatest variety. The comparatively small number of bacteria found in the small intestine has its explanation partly in the small amount of food that lodges there and partly, perhaps, in the bacteriolytic action of the succus entericus, which, though moderate, is appreciable. Wherever particles of transformed casein are found there will bacteria also be abundant, but with the exception of the lower ileum the small intestine does not harbor food-masses to any considerable extent. The epithelial cells are said to contain an antitryptic ferment and this passes to some extent into the succus entericus, where it is perhaps capable of exerting a restraining influence on that peptonization of proteid which is the first essential step toward putrefactive decomposition.

A satisfactory study of the products of the mixed fecal flora from normal nurslings has not yet been made. One fact, nevertheless, stands out, that on sugar-bouillon containing blood the volatile acid or acids produced give a molecular weight corresponding closely to that for acetic acid. The insignificant amounts of the higher volatile fatty acids points to the absence of considerable numbers of anaërobic putrefactive bacteria. In harmony with this is our observation that the Welch-Nuttal incubation test with rabbits does not produce the gas-liver from putrefactive anaërobes. The mixed fecal flora when grown on plain bouillon make indol, doubtless owing to the multiplication of colon bacilli.

If one makes a comparison of the bacteria of the digestive tract of infants fed on cow's milk with the flora of the digestive tract of breast-fed infants, many points of resemblance and also some typical and important differences are found. In general, the number of bacterial forms present is greater in the case of the bottle-fed infant than in the breast-fed infant, especially when the milk has not been sterilized or pasteurized. When sterilized milk is employed the increase in the number of bacteria in the digestive tract is dependent, at least in part, on the presence of anaërobic bacteria or facultative varieties capable of forming spores. Dr. Herter says that many of the bacterial forms found in the nursling's intestinal tract are also inhabitants of the intestine of bottle-fed infants, although in the case of the latter the organisms of the colon type predominate, so that the microscopic picture is Gram-negative instead of Gram-positive.

The products of decomposition in the intestinal tract of bottle-fed infants are said to be remarkably small in amount, as is the case in nurslings also. The large intestine of a normal bottle-fed infant contains merely a trace of indol or none at all. Only a moderate amount of volatile acid is obtained from the distillate of an acidified watery suspension made from any portion of the contents of the intestinal tract, and, of this, acetic acid forms by far the larger amount. This, Dr. Herter thinks, indicates that such bacterial processes of decomposition as occur within the intestinal tract are of a fermentative rather than a putrefactive nature.

After infancy the more varied diet increases the opportunities for the entering of many kinds of bacteria into the digestive tract, and, although individual variations are considerable, Dr. Herter describes conditions that are fairly typical for persons in good health and favorable environments. He says:

The Bacterial Conditions After Infancy.—During childhood and adolescence one sees a slow transition from the conditions of infancy to those of adult life. *B. bifidus*, although present, is much less numerous, and

other types are more numerous. Still the numbers of putrefactive anaërobes are small and putrefactive processes in the intestine are not active. This is shown by the presence of only a very small amount of indol and phenol in the feces, and, in the urine, by low ethereal sulphates and the absence or small amount of indican and phenol. The reaction with dimethylamidobenzaldehyd $((CH_3)_2N.C_6H_4.CHO)$ is slight or moderate—often so slight that its existence is questionable. During temporary derangements of digestion there may be an increase of the ethereal sulphates or indican, but this is very transitory.

Toward adult life great differences exist in the habits of different persons, and these are in a degree reflected in the nature of the bacterial processes of the digestive tract. In adult life the individual experiences new responsibilities, new dangers, an enhanced emotional life and often a larger proportion of indoor life and more sedentary habits. The dietary is apt to undergo an alteration in the direction of increased and frequently injudicious liberty and the use of tea and coffee. Also the use of tobacco and alcoholic drinks is either increased or begun. Sooner or later these things lead to slight derangements of digestion which manifest themselves clinically. One occasionally meets with persons of unusually robust physical and mental health in whom the bacterial conditions of adolescence persist until the fiftieth year, or longer. A large proportion of persons, however, by the time they reach the age of 50 present different physical conditions, although they are in no sense in a state of invalidism, but work hard and most of the time feel well. While in such persons the fecal flora shows nothing striking, it is usually not difficult to demonstrate that the number of putrefactive anaërobes in the intestine is larger than in healthy adolescents. In short, we find in middle life a large number of persons whose health is good or fair, in whom the putrefactive processes are distinctly more active than is the case with most younger persons of normal health.

These persons, though in good health, are not robust. A period of sustained hard work is followed by considerable mental and physical fatigue. Dining out and the use of alcoholic drinks are indulgences quickly followed by unpleasant consequences. Exercise out of doors becomes more and more a necessity. The individual is

conscious that it requires careful living to keep him in a condition compatible with the performance of his duties.

The main difference between the putrefactive conditions found at 50 and at 70 is that at the latter period they are a little more marked in their intensity and affect a much larger proportion of the population. The subjects in question at this later period of life are not ill, but in order to keep fairly well have to be very careful as to their habits of living. They are moderately anemic and easily develop slight disorders of digestion. They weigh less than formerly and, though they may still be well nourished in appearance, are conscious of losing strength from year to year. They are undergoing what is usually regarded as normal involution. It may be confidently asserted that the onset of senility may be distinctly accelerated through the development of intestinal infection in which the putrefactive anaërobes are prominently represented. I have observed this in cases where it has appeared certain that other toxic causes of premature senility could be excluded.

The methods of investigation employed by Dr. Herter in the pursuit of knowledge in connection with this subject are described at length. They relate in part to the study of the morphologic and cultural characteristics of the bacteria found in the digestive tract under different conditions, but in the main they deal with the products of the life activities of these bacteria when grown on different culture medium. Dr. Herter discusses the study of a microscopic field with the aid of the Gram stain, the isolation and identification of individual bacteria by means of plate cultures, the study of anaërobiosis by means of cultures and by animal experiments, the study of gas production, and such other procedures as are commonly carried out in the identification of bacteria. To quote Dr. Herter:

The appearance of the Gram-stained flora gives, as a rule, but not always, an indication of the dominant flora in the lower part of the intestine. One can not rely on it alone, but in connection with data derived from other methods it helps us to form a conception of the bacterial types present. In addition to the study of the mixed

fecal flora in the fermentation tubes, as a routine procedure, four flasks, each containing about 500 c.c. of medium, have been inoculated with a suspension of the mixed fecal flora and incubated seven days. The media employed have been peptone-bouillon, peptone-bouillon with calcium carbonate, sugar-bouillon and sugar-bouillon with calcium carbonate. Under the conditions prevailing in these flasks a large part of the growth has been anaërobic and a high degree of anaërobiosis has been maintained, owing in part to the formation of reducing products, such as hydrogen, incidental to the fermentative and putrefactive cleavages. It has been found in general that the anaërobes grow more abundantly in the flasks which were kept neutral by the presence of calcium carbonate. The chemical examination of the seven days' flasks has included two different series of procedures. The peptone-bouillon flasks were examined for hydrogen sulphid, methyl mercaptan, volatile fatty acids, ammonia, indol, skatol, phenol, alcohol and acetone. Quantitative determinations have regularly been made in the case of the volatile fatty acids, ammonia, indol, skatol and phenol. In the sugar-bouillon flasks the contents have been examined for alcohol and acetone, volatile fatty acids and the non-volatile organic acids. The molecular weights of the barium salts of the volatile fatty acids have regularly been determined. An interesting observation has been made that in the flasks containing calcium carbonate the molecular weights obtained for the volatile fatty acids have nearly always been somewhat higher than in the case of the molecular weights obtained from the volatile fatty acids of the sugar-bouillon flasks. This fact confirms the evidence of the microscopic fields and shows the greater abundance of the putrefactive anaërobes in the neutral flasks than in the sugar-containing flasks that are allowed to become acid. Methyl mercaptan has been determined by the isatin-sulphuric acid method. I have published previously the method used for the determination of indol and skatol and their separation by means of β-naphthaquinone-sodium-monosulphonate and the dimethylamidobenzaldehyd reaction. The chemical methods of studying the feces and urine are those that are fully described in the text-books relating to these subjects. To these known methods has been added the color reaction

of the filtered watery extract of the feces with Ehrlich's aldehyd and also the urinary reaction with this reagent.

The chemical products of intestinal fermentation and putrefaction, the individual susceptibilities as possible factors in determining clinical types of putrefaction, the types of chronic excessive intestinal putrefaction, and, finally, the therapeutic considerations which arise as the natural result of Dr. Herter's very careful study, are discussed by him as follows:

THE CHEMICAL PRODUCTS OF INTESTINAL FERMENTATION AND PUTREFACTION.

I shall use the word fermentation to designate the decomposition of carbohydrate and fatty substances and the word putrefaction to apply to the cleavages of proteid and allied substances. The products of putrefaction include the substances containing sulphur or nitrogen or both sulphur and nitrogen. The fermentative and putrefactive processes overlap in the sense that they furnish some products in common, such as carbon dioxid and volatile fatty acids, and, furthermore, they are linked by the fact that excessive fermentation in the digestive tract nearly always leads to excessive putrefaction. Of the products of fermentation the carbon dioxid acts mainly as a cause of flatulence in the stomach or small intestine. The acids formed—chiefly acetic and lactic—are irritants and may be exciters of vomiting and diarrhea. When in excess the acids may be excreted, unburned, and thus withdraw alkali from the tissues. It is possible that a mild degree of acidosis may thus result from fermentative processes in the intestine.

It is now well established that various molds and bacteria are capable of acting on media containing sugar in such a manner as to give rise to the production of oxalic acid. Dr. Helen Baldwin has shown that by prolonged feeding of dogs with large amounts of sugar a mucous gastritis is incited and that oxalic acid is present in the stomach and urine. It was also found that in media containing beef extract and sugar, oxalic acid was produced after inoculations with the contents of the stomachs of persons showing marked grades of oxaluria. Although gastric fermentation is not the chief source of oxalic acid in the body, it is possible that it may have an influence in causing the condition known as oxaluria.

When we turn to the consideration of the nitrogen-holding and sulphur-holding products of putrefactive cleavage, the scantiness of our knowledge comes into view with almost discouraging clearness. That putrefactive processes are attended by the formation of bases such as ammonia, amines, diamines (such as putrescin and cadaverin), cholin, neurin, sulphur compounds and various aromatic bodies, has been known many years and something has been learned, though by no means enough, about the media and the bacteria which determine the presence and proportions of these substances. When, however, we ask ourselves what we can safely say of the conditions under which such substances arise in the human intestines and of their pathologic effects, we are able to give in most instances only very inadequate answers.

Basic Substances.—Although ammonia is regularly formed in the course of putrefaction in the intestines, it is probably present in too small quantities to be toxic. The organism is well adapted to care for moderate quantities of ammonia which, as is well known, is united with carbon dioxid in the liver and elsewhere to form urea. It is possible, however, that ammonium butyrate may act as a local irritant in the intestine. Likewise we know nothing of any toxic action from methylamine or other alkyl amines. Cholin and, perhaps, neurin have been found in the intestinal tract in experiments on animals, but we lack positive evidence that they can under these conditions exercise their poisonous effects on the organism.

Putrescin and Cadaverin.—Although the study of the conditions under which putrescin and cadaverin are formed in the intestinal tract is of much biologic interest, there is at present little evidence that these diamines are ever formed in sufficient quantities in the human intestine to constitute in themselves factors in the production of states of intoxication. The association with cystinuria is a striking fact, and the further investigation of this condition will doubtless give us the explanation of the relationship between the production of diamines and the formation of cystin, if, indeed, there be any necessary relation.

Sulphur Compounds.—The sulphur compounds resulting from putrefactive decomposition in the intestines have received little attention from the standpoint

of their pharmacologic action. It is very difficult at present to form a just estimate of their importance in intestinal intoxications.

There is reason for thinking that the production of hydrogen sulphid in the digestive tract is of more importance to the organism than the formation of mercaptan. This gas is regularly formed in the intestines and its presence can be demonstrated in freshly voided feces. The mixed fecal flora, both in health and disease, produce hydrogen sulphid in cultures containing partially hydrolyzed proteids (bouillon). In health probably hydrogen sulphid is formed only in the colon and perhaps in the lower part of the ileum. There are, however, pathologic conditions in which it occurs in the stomach. It is not necessary to assume the presence of a pathogenic organism in these cases, as it is well known that *B. lactis aerogenes* and colon bacilli liberate it when growing in certain media. In marantic children I have found organisms capable of producing hydrogen sulphid in pepton-bouillon in the stomach and the first part of the small intestine; while in children dying of bronchopneumonia, such results were obtained only from the flora of the lower ileum and colon.

We have at present very little satisfactory knowledge of the influence of hydrogen sulphid on the organism in cases where the gas is liberated in the intestine. Senator and others have described poisoning by this gas. Among the symptoms which have been met with in such cases there have been prominent those pointing to disordered function of the central nervous system, including headache, dizziness, delirium, mental depression, drowsiness, stupor and collapse. Somewhat similar manifestations have been observed in experimental poisoning by hydrogen sulphid in animals and men.

AROMATIC PRODUCTS OF PUTREFACTIVE DECOMPOSITION.

Phenol and Cresol.—In some pathologic conditions attended by excessive putrefaction in the intestine these substances are found in the intestinal contents in amounts considerably above the normal amount, which is always small. But one never, however, finds them in large quantities—never so much, for example, as in the case of indol. Notwithstanding this, the quantity excreted in twenty-four hours in the urine as phenol potassium sulphate may be fairly high owing to the fact that

phenols are produced in the organism in the course of the metabolism of normal cells. In certain putrefactive cases I have found these substances in considerably greater amounts in the urine, but even here, however, it does not appear that the phenols can be regarded as important toxic agents, although it is likely that the continued absorption of moderate quantities from the intestine over a long period of time may harm the cells of the liver and other structures concerned with the pairing of phenol and sulphuric acid, especially if the cell protoplasm of the liver has previously been somewhat damaged.

Skatol.—This substance is formed in very small quantities from time to time in some normal persons and very abundantly in some persons suffering from excessive intestinal putrefaction. In persons with marked intestinal or nervous disorders I have occasionally found in the feces as much as 8 or 10 mg. of skatol in 100 gm. of feces. Usually the amount is much less than that of indol, but this rule is not invariable. Like indol, it is derived from tryptophan, but what are the conditions, bacterial and other, that determine its formation rather than the formation of indol, we do not at present know. I have found that the administration of skatol to monkeys by the mouth and by subcutaneous injections has been followed by the appearance of a substance in the urine giving the Ehrlich dimethylamidobenzaldehyd reaction and that the administration of 0.1 gm. of skatol to man has heightened the Ehrlich reaction in the urine. In most cases in which the feces contain considerable skatol the urine gives a strong reaction with Ehrlich aldehyd. Skatol behaves in the organism much like indol as respects its toxic properties, but it is somewhat less poisonous. There is seldom reason to attribute to it any definite pathologic effects. It is possible, however, that, like phenol, it may, under some conditions, play an auxiliary part with other substances in damaging living cells.

Indol.—Indol is not a product of tryptic digestion of proteids and probably can not be formed in the course of physiologic processes without the intervention of organized ferments such as bacteria. The indol produced in the intestine is, like skatol, derived from tryptophan. In early life the production of indol in the intestines is in general very slight and there are some older persons

also who, even while suffering from disorders of digestion, do not form indol. On the other hand, the production of considerable quantities of indol in the large intestine is a feature of many instances of intestinal putrefaction and in some cases the quantity formed is large. That indol may be absorbed in considerable amounts is shown by the appearance of large quantities of indican in the urine of persons in whom the intestine contains large amounts of indol.

While it is true that in general the aromatic compounds are resistent to oxidation, it is probable that whenever indol is introduced in moderate quantities into the organism of carnivorous and omniverous animals, a portion of it is burned completely in the body. It may be regarded as settled that the liver, muscles, intestinal epithelium and other cells normally exert a protective action to the nervous system in screening it from the effects of an injurious percentage of indol in the blood, by the ability of these structures to quickly bind any indol which comes to them. The differences in the observed toxic effects are probably dependent on inequalities in different persons in their ability to oxidize indol and to pair it with sulphuric acid. As to the effects of absorbed indol on the organism in disease, it is necessary to speak with caution, since there is no evidence that indol is the only toxic substance absorbed in those cases where it enters the organism from the gut.

The idea that the circulation of free indol in the blood may act in a depressing manner on the muscular structures is suggested by the rapid muscular fatigue which comes on in some persons who have suffered for a long period of time from a high grade of indicanuria. In some cases of excessive intestinal putrefaction in childhood associated with retardation in growth and abdominal distension there is clearly a poisoning of the muscular system. These children show signs of fatigue very rapidly, and in some cases where the condition has come on in early life they are slow in learning to walk. Their urine contains not only a large amount of indican, but a considerable quantity of phenol. It is likely that phenol in these cases plays a part in the muscular depression. Perhaps in some instances it is as much a factor in inducing fatigue as is indol.

INDIVIDUAL SUSCEPTIBILITIES AS POSSIBLE FACTORS IN DETERMINING CLINICAL TYPES.

Instances are many in which clinical experience has made it clear that two persons of approximately the same weight react differently to the same drug and do so regularly. Of individual human susceptibilities and reactions to the action of enterogenous poisons almost nothing is now known. Nevertheless, one can not fail to recognize the possibility that such individual susceptibilities and reactions may play an important part in determining the clinical manifestations of intoxications. It is well known to clinicians that there are some persons who promptly develop a widespread urticaria after indulgence in certain foods or drinks, such as shell-fish or strawberries or champagne. In some persons the indulgence in a single glass of champagne is followed within twenty-four hours by manifestations of gout. In others champagne causes headache and the excretion of increased amounts of uric acid.

The explanation of these different effects is to be sought in the individual cellular reaction of the patient rather than in the poison. There are probably many similar examples of individual susceptibility, but when we come to study the question in relation to processes found in the digestive tract we can not make close comparisons between different persons because we can not say what substances are being absorbed. We may know that a certain group of patients are alike in having intense indicanuria, but we can not say that the intoxications may not be different in these cases owing to differences with respect to the absorption of other substances than indol. Among half a dozen persons suffering from extreme indicanuria one suffers from headache, sometimes migraine-like; another is prone to lumbago; another perhaps has epileptic seizures; another has mental depression; another progressive muscular atrophy, and still another suffers from cyclic vomiting. There is good reason for suspecting that very similar bacterial processes in the digestive tract lead in one case mainly to digestive disturbances and in others, owing to a lesser sensitiveness in the digestive tract itself, to better absorption of poisons and the development of more remote consequences, such as acute arthritis, anemia or nervous disorders. While it is possible that these very different manifestations are always connected with different and

perhaps specifically different types of gastroenteric infection and intoxication, the possibility is not excluded that even such very different derangements may have much in common in their etiology. That the mental and emotional peculiarities of individuals have a large part in fixing the type of nervous reactions that occur in consequence of intoxications has become apparent to careful students of pathologic conditions.

TYPES OF CHRONIC EXCESSIVE INTESTINAL PUTREFACTION.

The variations in the clinical manifestations and pathologic accompaniments of chronic excessive intestinal putrefaction suggest that the etiologic conditions vary in different patients. The three types that I would suggest are:

1. The *Indolic Type* of chronic excessive intestinal putrefaction. This is marked by striking indicanuria and probably is due to members of the *B. coli* group.

2. The *Saccharo-Butyric Type* of chronic excessive intestinal putrefaction, which seems to be initiated chiefly by the anaërobic forms. In its simplest examples there is very little indol in the gut.

3. A *Combined Type*, or cases combining the characteristics of Groups 1 and 2.

Indolic Type of Chronic Excessive Intestinal Putrefaction.—In these cases the members of the *B. coli* group form indol in considerable quanties and they probably invade the small intestine in large numbers. The bacterial cleavages seem largely to replace normal tryptic digestion.

Provisionally we may classify here that type of chronic intestinal indigestion found in marantic children with large abdomens. In the treatment of these children much patience is necessary. At first their digestive processes must be improved. Carbohydrates should be greatly restricted and should be given as rice or Huntley and Palmer biscuits. The milk may be peptonized to promote its earlier absorption. Chicken, beef and mutton are permissible, but they should be finely divided. In a child 5 or 6 years old it may be advisable to give only two meals a day. Considerable benefit seems to follow daily irrigation of the colon, which facilitates the removal of the putrefactive products before they are absorbed. The children should exercise,

but should be spared fatigue. They should rest much. Because they stand cold badly, they do best in a mild climate during the winter. Improvement may be possible after several years of rigid régime. The retarded growth, however, is evident even at puberty. Some of these patients seem always susceptible to intestinal disorders, and may never become strikingly robust.

The Saccharo-Butyric Type of Chronic Excessive Intestinal Putrefaction.—In this type the seat of the putrefactive process is the large intestine and lower ileum. It is due to the activity of the strictly anaërobic butyric acid producing bacteria. Although our study is not yet exhausted it may confidently be stated that in many cases *B. aerogenes capsulatus* is largely responsible. With this form may be associated *B. putrificus* and possibly sometimes the bacillus of malignant edema, although often these forms are not found in cultures on any of the ordinary media.

The abundance of putrefactive anaërobes, especially of *B. aerogenes capsulatus,* gives a peculiar character to the intestinal contents. The organisms attack carbohydrates and proteids vigorously and butyric acid is formed from both, together at times with propionic, caproic or valeric acid. These acids are largely responsible for the odor of the stools. From proteids, besides these acids, hydrogen, carbon dioxid and perhaps methane are formed. As a consequence the feces have a low specific gravity and often a decidedly light color. The presence of hydrogen leads to the extensive reduction of bilirubin and other pigments. The Schmidt test with mercury bichlorid gives a strong pink color. The stools have an acid reaction, although the acids are neutralized in part by ammonia and other bases formed in the process of putrefaction. It is probable that the ammonium butyrate acts as an irritant to the gut, causing soft stools or diarrhea. Indol is absent or present in small amounts. Phenol occasionally is found in slight excess. In the urine the ethereal sulphates at times are excessive, although the reason for this is not clear. Mercaptan may be present in the feces as a trace; it also is found in cultures by means of the isatin-sulphuric-acid test. It has been noted that as the patient improves the mercaptan becomes less or disappears, but the explanation of this phenomenon is at present unknown.

In nearly all adults the *B. aerogenes capsulatus* is

present in the intestines in small numbers. It is possible that this organism is responsible for repeated slight attacks of intestinal putrefaction, although it may not in these mild cases lessen the duration of life. In some persons in whom a high grade of putrefaction is present, a condition of actual invalidism may be induced and life may be definitely shortened as a consequence of the intoxication.

The presence of ammonium butyrate may give rise to irritation of the intestine and this may be associated with an excessive desquamation of the epithelium, not only in the intestine, but in the mouth and stomach as well. We have evidence of this in the presence of a large number of nuclei in the feces, and it is well recognized that excessive desquamation of the lingual epithelium is associated with digestive disorders. The patients suffer from flatulence. They tolerate carbohydrates and acids badly, and are very liable to attacks of diarrhea after a meal of carbohydrates. Acids may be formed in the mouths of these patients through the influence of anaërobes. This adds to the irritability of the intestine. It is possible that in advanced cases the *B. aerogenes capsulatus* may invade the small intestine and there find sugar from which to form butyric acid, etc. After the carbohydrates are thus exhausted, the anaërobic forms in the large intestine set up putrefactive processes in the proteids which exist there.

It is also noteworthy that many patients who suffer from severe intestinal putrefaction are distinctly anemic. The first change in the blood seems to be a decrease in its volume; then the hemogloblin falls somewhat and finally the cells themselves are reduced in number. The grade of anemia varies extremely, from a moderate secondary anemia to the most serious grades of the progressive pernicious form.

The Combined Indolic and Saccharo-Butyric Type of Chronic Excessive Intestinal Putrefaction.—Examples of this type of intestinal putrefaction are common. There are many putrefactive anaërobes in the gut, and also a persistent and well-marked indicanuria, which is but slightly influenced by diet. The nervous symptoms are relatively prominent and appear early. They are emotional irritability and periods of mental depression; muscular or mental activity soon induces a striking fatigue. Later the blood disturbances may appear. Al-

though these patients have intervals of improvement that continue for months, on the whole the general tendency is downward. They become less robust and recuperate less promptly from every succeeding attack. They may run along for ten or fifteen years in a weak condition, with periods of slow improvement, and finally may present the picture of a pernicious anemia. In others the nervous symptoms increase and the patients may need treatment in a sanitarium or in an asylum for the victims of melancholia.

These various manifestations in different individuals may represent merely a differing reaction to the same poison. Whether the nervous system or the blood shall bear the brunt of the attack is determined by the relative vulnerability of these tissues in that particular individual. It is noticed also that under treatment one group of symptoms may improve quite independently of the other.

There is a more rapid advance of invalidism than is the case of either type (1) or type (2) alone. The atrophy of the fat and muscle and the blood changes are present, and perhaps also there are chronic parenchymatous changes in the kidney and liver as a result of the constant poisonous action.

THERAPEUTIC CONSIDERATIONS.

The difficulties that beset our efforts to control and modify excessive intestinal putrefaction are obvious. Although the cases arrange themselves in groups, everyone presents certain points of difference. Our experience is so incomplete that as yet our efforts are more or less experimental. Notwithstanding this, one may lay down rules for partial guidance that are based on certain principles, but a careful regard for individual traits is imperative.

The mild cases often show a rapid improvement and lose the evidences of putrefaction. The patient feels well, yet he can hardly be called normal, because he has deficient reserve power and will easily relapse to his former condition after an indiscretion in eating or excessive fatigue or worry. The long-standing cases improve slowly at best. The chemical products of putrefaction may be reduced in amount, but the symptoms often persist, and even under most favorable circumstances the patient is liable to frequent and protracted exacerbations.

The following principles must be regarded in treating all the three types of putrefaction: (1) Avoidance of continued reinfection that follows the ingestion of putrefactive bacteria with the food; (2) the promotion of prompt digestion and rapid absorption from the small intestine; (3) the reduction of the number of putrefactive anaërobes in the ileum and colon.

1. To avoid infection and reinfection the mouth must receive scrupulous care. Carious teeth and gingivitis must be treated carefully by the intelligent use of the tooth brush and of washes containing peroxid of hydrogen. In conditions of gastric atony a process of putrefaction begins in the stomach that normally starts in the colon. Gastric fermentation and putrefaction are controlled by lavage every day, perhaps best in the morning. The reduction of the number of bacteria here leads to lessened damage to the bowel at lower levels.

In the preparation of food ordinary cleanliness is very effective. It is probably better to use cooked food as much as possible. Fruit is not above suspicion, for Dr. Rettger has determined that the bacillus of malignant edema is commonly present on banana peel. This suggests the advisability of peeling all fruit that is eaten. Milk always contains a large number of bacteria and often some of the putrefactive forms, especially *B. putrificus*. The lactic acid formers abound, but their action is rather beneficial in that they antagonize other and harmful forms. Sterilization of the milk is of little value. Pasteurization or the ordinary boiling kills the lactic acid formers, but does not harm the spores of the putrefactive organisms. Cheese, except fresh home-made cheese, contains many putrefactive forms, and is best avoided, particularly inasmuch as many of these patients lack the protective action of the normal amount of hydrochloric acid in the stomach.

2. With rapid digestion and prompt absorption little pabulum for the putrefactive organisms reaches the colon. These processes are facilitated by measures that improve the secretory and motor functions of the stomach. Chief among these is proper mastication, which largely determines the ability of the body to utilize food. When large masses of meat are swallowed, they commonly appear in the feces. Comminution of food outside the body is not an adequate substitute, for the patient then loses the emotional stimulus to gastric secretion and

also the digestive action of the saliva itself. The administration of hydrochloric acid often helps for a time, but in long-standing cases, especially those of the combined indolic and saccharo-butyric types, it is of little use. Ferments, such as pepsin and pancreatin, are of doubtful value, although they can not be said to be always useless. Diastase gives better results, as it enables the patient to utilize more extensively the carbohydrates of the food. If, as often happens, the stomach is irritable, it is advisable to give small meals and to administer flaxseed or other demulcent before eating. The best pancreatic stimuli, aside from the quality of the chyme, are cheerful emotional accompaniments of eating, and rest, physical, mental and sexual. Prompt absorption is promoted by restricting the amount of food, especially of proteid food. Meat should rarely be eaten more than once a day.

3. To reduce the number of putrefactive organisms in the colon, one turns naturally to intestinal antiseptics. While these drugs may act efficiently on bacteria in the stomach, evidence of their continued action in the intestine is variable. Perhaps the salicylates are most likely to check fermentation and putrefaction in the stomach and small intestine. It is conceivable that certain oxidizing substances which are slowly dissociated, such as manganese bioxid, may reach the colon in time to liberate their oxygen there and thus, in part at least, remove the anaërobic conditions that obtain in this part of the intestine.

The use of laxatives may be followed by temporary benefit, in that they lessen absorption from the gut, as is shown by a decrease of the ethereal sulphates in the urine, after their use. They must, however, be given with caution, lest they increase the irritability of the bowel and lead to diarrhea and loss of strength. On the whole, they are useful in acute and subacute cases only.

There are certain very tempting methods which aim to substitute harmless bacteria for the putrefactive organisms, but more evidence is needed as to the value of this procedure. It is a common practice to introduce lactic acid formers in kumys and kefir and also in bacilac, a fermented milk introduced by Metchnikoff, which is free from yeasts. Irrigation of the colon two or three times a week is often followed by a decrease of the ethereal sulphates in the urine and by relief from symp-

toms, including both the mental symptoms and the anemia. This procedure is more efficacious in the saccharo-butyric and combined types of putrefaction.

PROGNOSIS.

In considering the prognosis in these patients, the duration of the condition is as important as its intensity. Better results are obtained in those cases induced by gross errors of life, the correction of which is followed by improvement or complete recovery. In a highly neurotic person the outlook is less hopeful. A protracted rest for two or three years, with careful attention to the principles of treatment laid down, offers the best hope of health.

The Influence of Food and of Epithelial Atrophy on the Manifestations of Saccharo-Butyric Intestinal Putrefaction.

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ONE HUNDRED AND THREE DEARBORN AVENUE.
CHICAGO.

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THE INFLUENCE OF FOOD AND OF EPITHELIAL ATROPHY ON THE MANIFESTATIONS OF SACCHARO-BUTYRIC INTESTINAL PUTREFACTION.

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In recent publications I have sought to establish the significance of the part played by anaërobic butyric-acid forming bacteria in chronic intestinal disorders and have advanced evidence to show that of these anaërobes, the *Bacillus aerogenes capsulatus,* or "gas bacillus," is the most important both on account of the frequency with which it is concerned and because of the intensity of its fermentative and putrefactive activities.[1] In assigning this place to the gas bacillus, I have not failed to recognize that other closely related, highly anaërobic bacteria, such as the bacillus of malignant edema and the *Bacillus putrificus* of Bienstock, are often associated with it. There are, in addition to these anaërobes, some butyric-acid forming aërobic bacteria which may thrive in the stomach and mouth, but there is no indication that in man they multiply abundantly in the large intestine, or in the small intestine, or that they are even moderately pathogenic for man. In some animals, as, for example, goats and pigs, large quantities of butyric acid are formed in the stomach by aërobic butyric-acid-forming bacteria taken in with the food and it appears likely that in them butyric acid is of importance in its relation to nutrition. These organisms, in fact, appear to be significant physiologically rather than pathologically, although we can not say that the continued per-

1. Bacterial Processes in the Intestinal Tract in Some Cases of Advanced Anemia, with Especial Reference to Infection with *B. Aerogenes capsulatus* (*B. Welchii*), Jour. Biol. Chem., ii, p. 1, August, 1906; see also Common Bacterial Infections of the Digestive Tract and the Intoxication Arising From Them, The Macmillan Company, 1907.

sistence of a butyric-acid type of decomposition of great intensity may not exert some detrimental influence on the duration of life.

The human condition which I have described as one of saccharo-butyric putrefaction[2] is characterized by the persistent presence of excessive numbers of anaërobic butyric-acid makers in the large intestine and probably also in the small intestine. In fact, the persistent occurrence of this form of putrefaction is an indication of an intestinal infection through the agency of anaërobes.

CHIEF CHARACTERISTICS OF THE SACCHARO-BUTYRIC TYPE OF INTESTINAL PUTREFACTION.

It is desirable for the purpose of the present paper to describe briefly the leading features of the saccharo-butyric type of intestinal putrefaction. The process in question is a chronic one. There are acute conditions in which there is a temporary increase of anaërobic butyric-acid makers, but in young individuals this condition is commonly of short duration—a few days or a few weeks—and is mainly of interest here as a forerunner of the more chronic type of infection. In chronic infection, the anaërobes have become adapted, at least partially, to the mucous membrane and its secretions. This is especially true of the large intestine where the putrefactive anaërobes may persist in very large numbers. The excessive numbers of anaërobic butyric-acid formers are readily demonstrable in the case of *B. aerogenes capsulatus* by (1) the large numbers of characteristic Gram-positive bacilli seen in the fecal fields; (2) the growth of characteristic colonies on anaërobic blood-agar plates with or without sugar; (3) the intravenous injection of fecal emulsions into rabbits incubated by the Welch-Nuttall method; (4) the subcutaneous or intramuscular injection of fecal emulsions into guinea-pigs, with the production of hemorrhagic edema at a distance as well as locally and by the spread of the bacilli (usually in pure culture) throughout the blood.[3]

2. I use the term putrefaction rather than fermentaton to indicate that butyric acid is formed from protein as well as from sugar and because the most harmful products are those derived from protein: at the same time the adjective "saccharo-butyric" is a reminder that butyric acid is formed from sugar.

3. I expect soon to publish exact data regarding the virulence of the heated and unheated fecal suspensions from a series of cases, as I believe the virulence of *B. aerogenes capsulatus* (in the human tract) varies almost as much as its numbers.

How far into the ileum the organisms establish themselves in significant numbers is not yet clear. While they are continually present in the large intestine in excessive numbers, it is probable that in many cases their presence in the small intestine is less constant and that the upper level of the small intestine to which they attain is variable, depending on the state of the secretion of the digested juices, on the character of the food and on the state of the epithelium of the intestinal tract. In one instance, I have obtained experimental evidence of the upward wandering of the *B. aerogenes capsulatus* to a level at least as high as the middle of the small intestine. This was in a dog in which Dr. Carrel had resected the pancreatic and biliary ducts. There was, however, in this case an acute gastroenteritis, apparently induced by an experiment in diet (which will be referred to later). A comparison with other animals shows that exclusion of the bile and pancreatic juice is not necessarily followed by this upward wandering of the *B. aerogenes capsulatus*, but that this is likely to occur under certain pathologic conditions. In human beings with chronic anaërobic infections, there is usually a diminution in the numbers of colon bacilli in the feces—perhaps even a complete disappearance—which probably points to a diminution of these organisms at higher levels. If the chronic infection has been of only short duration, the colon bacilli may still be well represented, and, indeed, this is the case in some infections of many years' standing.

In the saccharo-butyric type of intestinal putrefaction, there is an excessive production of butyric acid on an ordinary mixed diet. The amount of butyric acid recoverable in the feces depends in part on the proportion of acid that has been absorbed and is, therefore, greater, the conditions of diet remaining the same, when there is an increase in peristalsis from any cause, or when the epithelium has been temporarily or permanently denuded. It is usual in the saccharo-butyric type of putrefaction for the feces to have the odor of butyric acid, owing to the presence of a small amount of the free acid. This is especially noticeable on a diet rich in carbohydrates, but if the diet consists largely of meat the acid is neutralized wholly by ammonia or ammonia and other bases, and the odor of butyric acid is not pronounced. The quantity of butyric acid in one hundred

grams of dried feces may reach 0.5 grams in well-pronounced examples of this type of decomposition. The formation of butyric acid from carbohydrates is attended by the liberation of hydrogen, carbon dioxid and methane, and this gas production accounts for the intestinal flatulence which is so persistent a feature in marked instances of the affection. The quantity of hydrogen sulphid produced is small and in several instances the quantity of hydrogen sulphid bound by the proteids of the feces has not been in excess of that observed in normal persons on a similar diet.

Often the expelled gases have a sickening odor, exactly like that which emanates from a rabbit that has been injected with a pure culture of *B. aerogenes capsulatus* and then incubated. The presence of gas gives a light color to the movements which are often unformed and soft and float on water. Through the active reducing agency of the putrefactive anaërobes there is a conversion of bilirubin into hydrobilirubin, the presence of which may be inferred from the pink or red color developed on the addition of a saturated solution of mercuric chlorid. This reaction is frequently intense. It fails, however, in those conditions in which the bile is excluded from the intestinal tract.

Another unusual reaction, dependent on some modified form of bile pigment (perhaps urobilinogen) is that which is manifested when an acid solution of dimethylamidobenzaldehyd is added to the filtrate prepared from a fecal suspension. The intense cherry-red color so obtained is like that yielded by indol. Hence in order to determine whether the reaction is due to urobilinogen it is necessary first to remove by distillation any indol which may be present. It is very common to find indol in the intestinal contents of persons suffering from chronic saccharo-butyric putrefaction, but the quantity may be small, and I have met with instances in which the feces contained a great excess of anaërobes, chiefly *B. aerogenes capsulatus,* but from which only traces of indol could be recovered, while the urine on repeated examination failed to contain indican.

I consider that two conditions have to be fulfilled in order that a saccharo-butyric putrefaction should be unattended by indol formation. The first is that the dominant anaërobe should not be an indol producer. This is frequently the case with *B. aerogenes capsulatus.*

The second condition is that the colon bacilli, of which the intestine almost always contains many that are indol formers, shall not take part in the process of putrefaction. In other instances there is an abundance of indol produced, and consequently a strong reaction for indican in the urine. The explanation of the indol putrefaction must be sought either in the indol-producing properties of the anaërobes concerned (properties which they sometimes possess in a very high degree) or in the active cooperation of indol-making colon bacilli in the putrefactive process. I have met with instances in which the colon bacilli have been so few, both in the feces and in material obtained by catharsis, that I have questioned whether they could have any considerable part in the indol production.

In this connection I would call attention to two facts relating to indol production which I think are not recognized. One is that when the bile and pancreatic juice are experimentally shut out from the intestine there may be a large production of indol in the upper half of the small intestine as well as lower down. The second point is that indol production may occur in the small intestine in the presence of a distinctly acid reaction to litmus, this reaction being due mainly to the presence of free fatty acids.[4] Further reference will be made to this point in discussing the influence of the fats on manifestations of saccharo-butyric putrefaction.

THE PRESENCE OF SKATOL.

In many instances of pronounced saccharo-butyric putrefaction, skatol has been present in the intestinal contents. Contrary to the general belief, I have found that skatol is very often absent from the intestinal tract of normal people. I have never, under any circumstances, met with it in considerable quantities; that is to say, in quantities comparable to those in which one finds indol in cases of severe putrefactive decomposition, but the largest quantities I have observed (10 mg. in 100 gm. of fresh intestinal content) have been obtained from persons suffering from severe or long-standing forms of intestinal putrefaction.

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4. It is possible that in such cases there are minute portions of the intestinal contents that give a neutral or even slightly alkaline reaction to litmus, while the reaction of the contents as a whole is decidedly acid. This would explain the formation of indol, hydrogen sulphid and some of the putrefactive substances.

In many of these instances, putrefactive anaërobes have been extremely abundant, and I have come to regard the presence of skatol as a usual accompaniment of the saccharo-butyric variety of intestinal putrefaction, although I admit that in small quantities skatol may be found where there is certainly no long-standing or intense decomposition of this sort. I have found it very difficult to determine the conditions under which skatol is produced by putrefaction rather than indol. In the study of individual putrefactive anaërobes I have found three which gave rise to considerable quantities of skatol on pepton-bouillon. These were certain varieties of the bacillus of malignant edema, some forms of *Bacillus putrificus,* and a butyric-acid-producing anaërobe sent to me by Dr. Theobald Smith.

I believe that skatol is only rarely formed, if ever, by the *B. aerogenes capsulatus* acting on pepton-bouillon. For these reasons it seems to me not impossible that when we find considerable quantities of skatol in the feces in the course of saccharo-butyric putrefaction this must be attributed to some associated anaërobe or to some special symbeosis rather than to *B. aerogenes capsulatus.*[5] On the other hand, these considerations explain why it is that skatol is not always present even where there is pronounced saccharo-butyric putrefaction.

In many of the cases where skatol has been found in relatively considerable quantities, indol has been present in smaller quantities or not at all.[6] The ethereal sulphates of the urine are generally increased whether indican be present in the urine or not.

The presence of mucus in the feces in excessive amounts is a common occurrence. It is usually present in moderately excessive rather than largely excessive amounts and in rather small strings intimately intermingled with the feces. But frequently relatively large quantities may be found on the surface in some places, while in other parts there is no excess. Commonly, too, the microscope shows an abundance of epithelial cells

5. It is difficult to form a judgment on this point which takes account of the nutrient media found in the human intestine in these cases.

6. The presence of considerable skatol in the intestinal tract is apt to be associated with a strong reaction to dimethylamidobenzaldehyd. This reaction is strong, however, in all persons who eat meat to excess and such excessive use of meat is probably the explanation of the reaction in some persons with putrefactive disorders.

in the feces, often associated with mucus, but sometimes independently distributed through the contents of the intestine. In some instances, particularly where there has been associated streptococcal infection, I have found an abundance of spheroidal masses of nuclear substance which I have suspected to represent leucocytes, but some of which were probably nuclei derived from desquamated epithelial cells.

In the early stage of chronic infection with putrefactive anaërobes constipation is common. This condition is, however, varied from time to time by diarrheal seizures in which the gas-holding intestinal contents are discharged in large quantities. I attribute the constipation of this period to diminished peristalsis, due to impaired innervation of the gut and diminished secretion of bile and pancreatic juice. It is often possible, even in this early period, to determine that there is impaired secretion of free hydrochloric acid into the stomach, and it appears likely that with this there is associated a diminution in the intestinal secretions.

At a later stage of the affection, usually after it has existed several years, diarrhea is of frequent occurrence and is significant as a factor in bringing about a distinct decline in weight and strength. Usually the diarrheal movements consist largely of coccal forms, colon bacilli and anaërobic putrefactive organisms. Relatively slight indiscretions in diet suffice to bring on diarrhea. This sensitiveness I attribute to a chronic condition of congestion of the small intestine with some associated desquamation of epithelium—a condition in which slight irritation brings on a temporary increase in the local congestion and an increase in peristalsis.

Dr. Houghton[7] has lately made what I regard as a significant observation in a patient with pernicious anemia. The patient suffered with very marked manifestations of intestinal putrefaction (which, though not so described, appear to me to point unmistakably to a chronic infection with anaërobes) which it was attempted to relieve by an operative procedure which should permit free intestinal lavage.

At operation "the peritoneum resembled one subject to a low grade of inflammation without exudate. The

7. Apparent Recovery from Progressive Pernicious Anemia Following Cecostomy and Colonic Lavage, The Journal A. M. A., June 29, 1907, p. 2186.

color was darker than normal, the vessels injected, and that tissue itself very easily torn." I have long suspected that such a condition is a common occurrence in persons suffering from advanced forms of chronic excessive intestinal putrefaction, and now believe such chronic congestion will come to be recognized as a pathologic factor in many such cases.

It is not my intention to enter here on a full discussion of the clinical manifestations of chronic saccharo-butyric putrefaction, but rather to emphasize such biochemical features of the condition as are at present recognizable. I wish, however, to refer briefly to certain derangements of the nervous system, the blood and the general nutrition that may be regarded as characteristic of the affection.

CLINICAL SIGNS OF EXCESSIVE PUTREFACTION.

Among the earliest signs of excessive intestinal putrefaction is a state of debility which is usually described as neurasthenic. The patients are indisposed for work, especially in the mornings, the tension of the pulse is low and fatigue sets in quickly. The extremities are usually cold, and it is only after vigorous exercise that a better distribution of blood occurs. It appears likely that these symptoms are in a measure dependent on anemia of the nervous system, which in its turn is referable to intestinal irritation, due to the micro-organisms concerned with the infection. These neurasthenic symptoms gradually increase and may ultimately be associated with considerable mental depression, especially in those cases where the saccharo-butyric type of putrefaction is associated with the formation of considerable quantities of indol and the excretion of indican in great excess. The pulse is commonly faster than normal in uncomplicated cases.

The influence of chronic saccharo-butyric putrefaction on the blood I have discussed elsewhere, especially in its relation to severe anemias. It is only necessary to say here that the long persistence of excessive saccharo-butyric putrefaction due to the *B. aerogenes capsulatus* is followed by some degree of anemia and that in some instances this anemia is extreme, even presenting the indications of the pernicious type of the disease. While I have been unable to bring forward convincing proof that the severe forms of anemia observed in cases of

chronic excessive saccharo-butyric putrefaction are due exclusively to the agency of putrefactive anaërobes, I consider that certain clinical relationships which have been observed and the marked hemolytic action of the *B. aerogenes capsulatus* point to the latter organism as the one chiefly concerned in bringing about a degree of blood destruction which can not be adequately compensated by the blood-forming organs. A not infrequent association of chronic infection of the intestinal tract with the gas-bacillus is an infection with streptococcal forms, and where this exists it is not possible to say to what extent the blood destruction is due to this associated infection, since some of these streptococcal forms possess a hemolytic action. Not rarely patients develop a slight degree of cyanosis, especially noticeable in the extremities during cold weather or early in the morning. Even in the absence of distinct cyanosis the blood may have a darker appearance than normal.

The general nutrition of patients with chronic saccharo-butyric putrefaction gradually suffers. The persistent character of the diarrhea in the advanced stages of the disease contributes largely to this result, but even where exhausting diarrheas are absent nutrition is apt to fail. The nutritive failure is shown in subcutaneous atrophies of fat and connective tissue and gradual diminution in bulk of the muscles, which sometimes undergo fibrillary tremors, as in progressive muscular atrophies. These are changes which, for the most part, are to be expected in old age, and it is true that a premature senility is a feature of chronic saccharo-butyric putrefaction.

Having sketched the leading clinical characteristics of chronic saccharo-butyric intestinal putrefaction, I wish to speak of the influence of the chief kinds of foodstuffs on the course of the fermentative and putrefactive aspects of the process. While there are still many questions regarding the influence of foods on intestinal putrefaction from butyric-acid forming anaërobes to which no answer can yet be given, there are facts of theoretical and practical interest which stand out so distinctly as to merit recording.

THE INFLUENCE OF CARBOHYDRATES.

The putrefactive anaërobe which attacks sugars with the greatest activity is *B. aerogenes capsulatus*. This organism decomposes dextrose, lactose and saccharose.

Gas-production is in each case rapid and abundant. Moreover, boiled starch is inverted, though not quickly. The presence of at least a trace of sugar appears necessary to initiate the growth of this anaërobe, which does not multiply on sugar-free media containing proteins or proteins and starch. The gas formed through the decomposition of sugar is mainly hydrogen, the proportion of this gas to other gases formed being 2:1 or 3:2. Of the other gases formed, carbon dioxid is the chief, but a little methane is also made. Butyric and propionic acid are freely formed and acetic and even valerianic acids may probably be produced in traces.

I consider it noteworthy that patients with chronic saccharo-butyric putrefaction tolerate carbohydrates badly. In the early stages of the affection a meal rich in carbohydrates, whether in the form of sugars or starches, appears to make little difference (although there is some evidence that the sugars give rise to the most disturbance) causing moderate intestinal flatulence, abundant but formed stools, smelling of butyric acid, and perhaps also an increased frequency in micturition.

There is often a sense of drowsiness or dulness after an excessive carbohydrate meal, but seldom headache. In later stages of the affection, when the general nutrition has begun to fail and the patient falls below the normal weight, there appears an increasing sensitiveness to carbohydrate food which shows in the prompt occurrence of diarrhea and intestinal flatulence after any excess. Indeed, there comes a time when carbohydrates, even in amounts that would be called physiologic and moderate for a normal man, cause distress from gas and soft, ribbony stools, if not diarrhea.

This abnormal sensitiveness may be so highly developed that diarrhea becomes a frequent occurrence and through its frequency makes further inroads on the weight and strength of the patient. Under such circumstances a pronounced restriction in the quantity of carbohydrates becomes imperative, for every infringement of a rigid dietary is promptly followed by increased peristalsis, abdominal discomfort, unformed movements or watery diarrhea. Sometimes diarrhea promptly follows the error in diet, in the course of a few hours, but more often there elapses a period of twelve to eighteen or twenty-four hours. In the period following the ex-

cessive use of sugars or starches and before the onset of unformed movements, the subject of this affection may become lethargic or even prostrated to such an extent as to feel quite indisposed for work or exercise. I am disposed to attribute this temporary state of lethargy (which may be only an exaggeration of the habitual condition) to the increase in the chronic congestion of the intestinal mucosa, which, as already mentioned, I assume to be a usual feature of the pathologic process in its advanced stage.

I consider the clinical phenomena just described as best explicable on the supposition that there has been an upward invasion of the small intestine by the gas-bacillus, although at the present time I have no proof based on early autopsies that this is actually the case in man. The presence of excessive numbers of gas-bacilli in the feces in diarrheal stools gives no positive indication of the level of the intestine at which they have actively multiplied, but in connection with the free formation of gas in the small intestine after carbohydrate food makes it reasonable to believe that these organisms are actively engaged in fermentative decomposition above the ileocecal valve, since no other bacteria found in the stools of these patients possess a gas-making capacity at all comparable with that of *B. aerogenes capsulatus*.

There is probably little simultaneous bacterial attack on the proteid food in the small intestine, for the gas-bacillus acts preferentially on the carbohydrates of the food. Nevertheless, free multiplication on carbohydrates prepares suitable conditions for subsequent putrefaction, as the gas-bacillus decomposes proteids more actively when carbohydrates gradually become scarce. Unless, therefore, the intestine be relieved of its proteids by the occurrence of diarrhea, gas-bacillus fermentation in the small intestine facilitates putrefaction at lower levels.

The maintenance of a strongly acid reaction is antagonistic to putrefaction, but alkali may be derived in sufficient quantity from the succus entericus of the pancreatic juice to neutralize such volatile fatty acids as fail to be absorbed. When the reaction of the intestine approaches neutrality to litmus the decomposition of proteid begins. This proteid decomposition yields both butyric acid and ammonia, and the latter may be suffi-

ciently abundant to establish a neutral or even slightly alkaline reaction.

In the advanced stages of chronic saccharo-butyric putrefaction there is developed a marked sensitiveness of the digestive tract to acids, which may lead to diarrhea. The digestive tract may become so intolerant of acids that ordinary moderate quantities of kumyss or bacillac or zoolak cause increased peristalsis or diarrhea owing to the lactic acid they contain. An equal volume of water may stimulate peristalsis, but does not cause diarrhea. The intolerance to fruits which exists in these patients is due in part to the organic acids they contain, but in part, of course, to their content of carbohydrates which yield butyric and other volatile fatty acids. Indeed, the intolerance of this group of patients to carbohydrates in general is probably to be interpreted as an excessive sensitiveness of the mucous membrane of the small intestine to the organic acids formed from carbohydrate cleavage.

It is perhaps hardly necessary to add that this sensitiveness (due to chronic inflammation) expresses itself also in an intolerance for mechanical irritants, such as cellulose, the seeds of berries, etc. In some instances of this affection the mouth quickly develops an acid reaction from the use of carbohydrates. This is, however, due to other varieties of bacteria than the gas-bacillus and does not appear to be a constant occurrence. Moreover, this excessive acidity of the mouth after taking sugar may be observed in persons who do not show any definite indications of the saccharo-butyric type of intestinal putrefaction.

INFLUENCE OF FATS.

The effect of fat on the course of the saccharo-butyric process in the intestine has received little consideration and is at present only imperfectly understood. The fact that neutral fats are relatively resistent to the cleaving action of bacteria leads one to suppose that they play an unimportant part in digestive disturbances characterized by excessive fermentation and putrefaction. On the other hand, there is clinical evidence that in many disorders of digestion the fats are not well tolerated. The chief manifestations of such intolerance are:

1. A tendency to nausea or regurgitation (especially in children) or even vomiting.

2. Abdominal discomfort referred to the intestine.

3. An impaired absorption of fat from the intestinal tract.

Any or all of these manifestations may be observed in cases of saccharo-butyric putrefaction after a meal rich in fats, but none of them can be regarded as in any way characteristic of the process. It should, however, be stated that, although exact fat metabolism experiments are as yet wanting in cases of well-defined saccharo-butyric putrefaction, there is considerable clinical evidence to show that even where moderate quantities of fat are ingested many persons suffering from this putrefactive disorder show a larger percentage of neutral fats and fatty acids in the feces than is the case with normal persons on a similar diet. This holds true even where there is no increase in peristalsis, to which diminished fat absorption might reasonably be ascribed. The necessity for accurately conducted metabolism experiments in such cases is evident.

In relation to the saccharo-butyric type of putrefaction there are two questions in reference to the destiny of the fats which arise prominently: First, how do the putrefactive anaërobes act on neutral fats in regard to their cleavage into fatty acids and glycerin, and, second, what is the influence of the fats on the anaërobic putrefaction of proteids?

THE TECHNIC.

In order to determine the fat-splitting action of various intestinal bacteria on neutral fats, a number of observations were made on media containing a sample of olive oil containing less than 1 per cent. of free fatty acids. In the earlier experiments made the medium consisted of 50 c.c. of peptone bouillon, 20 gm. of olive oil, 20 gm. of ground boiled beef, 100 c.c. of water and a large excess of calcium carbonate. A flask containing this medium was inoculated with fecal emulsions containing an abundance of *B. aerogenes capsulatus*. This fecal material was from a dog in which the biliary and pancreatic ducts had been resected and which showed evidence of greatly excessive intestinal putrefaction. A second flask was inoculated with a fecal emulsion from a dog with a shortened large intestine, but otherwise normal, fed on meat. A third flask was inoculated with human fecal emulsion containing an abundance of *B. aerogenes capsulatus*. The period of incubation was forty hours. The first flask contained 45 per cent. of free fatty acids, the second 37 per cent., and the third 29 per cent.

As it was desired to extend these observations to a greater variety of bacteria, another series of cultures was made. In this series the medium so employed was, for the sake of convenience, used in test-tubes instead of flasks. Each test-tube contained:

Olive oil	2 gms.
Pepton-bouillon	7 c.c.
Boiled beef (ground)	5 gms.
Calcium carbonate	1 gm.

In addition to the control tubes, tubes inoculated as follows were placed in the incubator: 1. Tubes containing fecal suspensions of bacteria, among which *B. aerogenes capsulatus* was abundant, the fecal material having been heated to 80 C. for 20 minutes to destroy vegetative forms; 2. heated fecal suspensions from another subject, containing *B. aerogenes capsulatus*, but in smaller numbers (this material was also heated to destroy vegetative forms); 3. fecal suspensions from normal breast-fed child, unheated; 4. *B. coli communis* (New York Health Board stock); 5. *B. putrificus* (Bienstock) obtained from Kral.

The repeatedly sterilized media, after inoculation with the above material, were incubated for 40 hours. In all cases there was some separation of fluid into layers with an oily layer of varying thickness at the surface. The inoculated tubes also showed a varying quantity of yellowish, cream-like material. The tubes inoculated with human fecal emulsions all had a pungent putrefactive odor with a somewhat sickening sweetish quality. The odor from the culture-free material from the breast-fed child was slight and rather ammoniacal; the odor from the *B. coli* inoculations was faint and slightly putrefactive; the odor from the *B. putrificus* inoculations was moderately putrefactive with a slightly sweetish quality and not ammoniacal.

After incubation the fat was extracted (though not completely) by repeated shaking with ether. The ethereal extract was filtered through absorbent cotton to remove whitish particles suspended in the extract. The ethereal extract was then washed with about one-quarter its volume of water to remove acids other than fatty acids. The ethereal extract was placed in weighed vessels, evaporated to dryness in the water oven and weighed, and then dissolved in boiling alcohol and titrated with a decinormal alcoholic solution of potash with phenolphthalein as an indicator. In cases where some insoluble material unavoidably found its way into the ethereal extract, the vessels were dried and weighed again for the correction of this error after throwing out the substance soluble in ether. The results showed that in the tubes inoculated with human fecal emulsions there had been a high degree of fat-splitting in one case 72 per cent.; in a duplicate, 59 per cent.; in a second case, 77 per cent. In the tube inoculated with the fecal extract from the breast-fed child the percentage

of fatty acids was 4.2 per cent. In the tubes inoculated with colon bacilli and with *B. putrificus* alone, the quantities of fatty acids recovered were less than 2 per cent.

THE RESULTS OBTAINED.

These results indicate that the human intestine may contain spore-forming bacteria capable of splitting olive oil to a very large extent. In the case of the human material employed for inoculation, it is reasonable to suppose that the spore-forming putrefactive bacteria were responsible for the high degree of fat-splitting observed. This fact is the more noteworthy, as previous observers have found that the human feces in twenty-four hours split the fat from butter or milk to a considerably less extent (8 to 12 per cent. in experiments by Fr. Müller). It has been supposed by some writers that the high degree of fat-splitting observed in the human intestine, despite the exclusion of the pancreatic juice and the bile, may be in part referable to a vicarious secretion of pancreatic enzymes into the small intestine.

In view of the results above reported, this explanation appears to me quite unnecessary to account for the degree of fat-splitting that has been observed in human subjects from whose intestinal tracts the biliary and pancreatic secretions have been shut out by disease. In this connection I may say that in a dog from which the pancreatic juice and the bile had been completely shut out, a high degree of fat-splitting was observed above the middle of the large intestine where putrefaction was extremely active. At the present time I have not made observations with pure cultures of *B. aerogenes capsulatus*, but this organism as well as *B. putrificus*, which in the experiments just noted formed so little fatty acid, is liable to grow poorly in the medium employed unless this has been enriched with substances of intestinal origin.

In regard to the influence of fats on putrefaction in the human intestine, I know at present of no reliable clinical observations. I can only say on this point that patients on a mixed diet containing fat, who have shown an excess of ethereal sulphates and of indican in the urine, have shown a diminution in the excretion of these putrefactive products when the carbohydrates of the diet have been in a considerable degree substituted by fats of low melting point (that is butter, milk or meat fat). One can merely say, therefore, that moderate

quantities of fat, when taken with proteids, probably do not increase the intestinal putrefaction in a significant way. It is possible, however, that the case may be different where very large quantities of fat are taken together with proteid. The introduction of large quantities of fat into the intestinal tract may confidently be expected to hinder mechanically the resorption of proteid food, and on this account to favor putrefactive decomposition if considerable numbers of putrefactive bacteria be present.

The following experimental observation may be recorded as bearing on the question of the influence of large quantities of fat on intestinal putrefaction where the bile and pancreatic juice have been effectively excluded from the duodenum.

A dog weighing 7 kilos was subjected by Dr. Carrel to an operation for resection of the bile duct and pancreatic duct. The animal recovered wholly from the operation, though developing jaundice and cholemia. Six weeks after the operation, the animal being in apparently good health, it was subjected to experiments in diet. The first of these experiments consisted in placing the animal on a diet of milk and bread. On this diet the feces were extremely fatty and had a strong odor of fatty acids, especially of butyric acid. Distillates from the intestinal contents showed only the faintest trace of indol, no skatol and no phenol. The urine, on repeated examinations, was entirely free from indican. This animal received two quarts of milk daily and varying, though considerable, quantities of bread. On this diet the animal was lively and apparently well. After a period of two weeks it was placed on a diet consisting of one pound of meat with from one to two ounces of meat fat. The feces were either neutral or after a few hours slightly ammoniacal. They contained an abundance of fat. Micro-organisms of the *B. aerogenes capsulatus* type were moderately but not extremely abundant, but were more numerous than in the milk feces. Distillates from the feces gave moderate indol reaction, a trace of phenol and no skatol. The urine regularly gave strong indican reaction. The animal appeared entirely well on this diet, which was maintained for over two weeks.

It was then placed for six days on a diet of ½ lb. of meat daily. On this diet the feces were scantier than before, lighter in color and firmer. Distillates from the feces showed the presence of indol in considerable amounts, no skatol and merely traces of phenol. The indican of the urine was considerable, although perhaps somewhat less than when on a diet containing one pound of meat daily. The observations made up to this point, therefore, indicated that the animal did well on the

diet of milk and bread, on the meat diet containing considerable fat and on the more restricted meat diet. Immediately following the period of restricted meat diet, the animal was given one-half pound of meat and in addition one-half pound of meat fat. The meal was given in the morning. Early the following morning, within twenty-four hours, the animal died. The cage was found very much chewed and this was taken to indicate that the animal had been in pain. There was neither diarrhea nor vomiting.

At autopsy it was found that the entire digestive tract, especially the small intestine, was intensely congested, and there was associated edema of the lungs, making it probable that death was induced by heart failure from a pathologic accumulation of blood in the portal area. The entire small intestine was filled with a grayish material with an odor suggesting fatty acids and with a slightly sweetish quality. The duodenum and upper jejunum contained large quantities of mucus, in part intimately mixed with the fatty contents and in part lining these portions of the intestines. Without entering into details, it may be stated that the intestinal contents even above the middle of the small intestine and in the upper part of the jejunum contained indol in abundance, as shown by the distillates from even small quantities of this material when tested by the dimethylamidobenzaldehyd reaction and the β-naphthalquinon-monosulphonate test. Phenol was detected only in traces. Hydrogen sulphid was also abundantly formed.

The material obtained from the upper part of the small intestine where this intense putrefaction was going on was shown to contain *B. aerogenes capsulatus* in abundance and small doses of it proved characteristically fatal to guinea-pigs when subcutaneously injected. The lower part of the intestine also contained indol in great excess. It is to be regretted that the many studies which it was necessary to make on this animal within a short space of time made it inconvenient to make a quantitative determination of the indol for the entire digestive tract. The indol reactions obtained in small quantities of fecal material were, however, more intense than from similar material on the earlier diet. The indican reaction of the urine was extremely intense.

Whatever interpretation may be placed on the occurrence of death after the addition of fat to the meat diet, there can be no doubt whatever that the enormous increase in intestinal putrefaction which was observed in this case was dependent on the addition of so large a quantity of fat to the meat, since in preceding periods a diet consisting of moderate quantities of fat and twice as much meat daily gave rise to no symptoms whatever. It appears that in this instance the addition of the abun-

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We are almost without knowledge regarding the influence of the constitution of different proteins on the decomposition products that result from the action of putrefactive anaërobes, and this gap is one which it is very desirable to fill by future studies. Proteins which, like casein, yield tryptophan abundantly, may confidently be expected to yield indol freely in the presence of indol-forming bacteria.[9] The chemical conditions under which putrefactive anaërobes give rise to skatol rather than indol are, as already mentioned, still unknown. The absence of the tryptophan nucleus in gelatin explains the failure of this substance to yield indol on putrefaction. A better knowledge of the putrefactive products from casein, from egg-albumin, from serum-albumin, from myosin and from gliadin (each of which yields distinctive proportions of various amino-acids on hydrolysis) is likely to prove helpful in enabling physicians more intelligently to control various types of putrefactive decomposition.[10]

One fact of practical significance stands out plainly with reference to the use of proteid food in cases of saccharo-butyric putrefaction, namely, that it is very common in this condition to find in the feces considerably more unutilized proteid than is found in normal persons of the same weight and on the same diet. There is, in other words, a diminished power of resorption of proteins attributable, I believe, to the slight or marked chronic enteritis of which I have found evidence in cases of chronic infection from *B. aerogenes capsulatus*.

In consequence of this diminished power of absorption, considerable proteid finds its way into the region in which the putrefactive anaërobes are most numerous —the lower ileum and the colon. This condition calls for notice by the physician and makes it incumbent on him to endeavor to reduce the proportion of unabsorbed proteins that find their way into the colon. There are three principal ways in which this improvement may be attempted with reasonable hope of success:

9. Some bacteria incapable of forming indols are unable to make this substance even when they are furnished with tryptophan. I have found this to be true of the bacillus of typhoid fever.

10. It appears especially desirable to study the putrefactive products derived from fish by the action of the intestinal anaërobes since persons exhibiting the signs of well-marked saccharo-butyric putrefaction seem especially susceptible to exacerbations after eating fish, sometimes even when the quantity taken is very small.

1. By reducing the quantity of the protein of the food.

2. By alternating the character of the protein ingested.

3. By means which, like physical and mental rest, improve the absorption of foodstuffs and at the same time diminish the protein requirements of the organism.

On the other hand, there is no more certain way of increasing the multiplication of the putrefactive anaërobes in the colon than by permitting the use of a large quantity of protein, provided the carbohydrate food be not simultaneously reduced. But, since the putrefactive anaërobes of the intestine, with the exception of *B. putrificus* (Bienstock), do not energetically decompose native proteids in the absence of carbohydrates, it is permissible for short periods to give a generous allowance of protein food,[11] if at the same time carbohydrates be absolutely excluded. Under these conditions an amount of meat can be taken with diminishing intestinal putrefaction, which, under the free use of carbohydrates, would certainly lead to an increase in the putrefactive products in the intestine and in the urine.

In all severe cases of excessive saccharo-butyric putrefaction a strict diet of egg-albumin, minced beef and water may be recommended from time to time for a period of forty-eight hours, with considerable confidence that it will cause a diminution in the formation of putrefactive substances and a coincident alleviation of the various symptoms liable to attend this process. This holds true, notwithstanding that in general the formation of indol is more likely to occur on a diet containing meat protein than on a diet containing milk. The objection to milk in severe exacerbations of saccharo-butyric putrefaction arises from the sugar which it contains. If on a meat and egg-albumin diet the indican does not diminish in consequence of better resorption and owing to some inhibition in the multiplication of anaërobic bacteria, then milk preparations should be tried in which the sugar has been removed by lactic acid fermentation.

In any consideration of the putrefaction of proteids in the intestine through the agency of *B. aerogenes capsulatus* or other anaërobes, it is desirable to touch on

11. Probably best in the form of egg albumin and minced beef with the addition of gelatin.

the therapeutic use of certain bacterial antagonists which have lately been somewhat insistently recommended in disorders of digestion. The idea has long been current that the fermentation of carbohydrates has an inhibitory action on putrefaction owing to the acids formed.[12] This idea is justified in so far as it is true that the presence of considerable free acid is in general unfavorable to putrefaction, which proceeds most rapidly where there is available alkali for the neutralization of acid. But, as pointed out in the preceding pages, it is not impossible for putrefaction to proceed energetically in spite of the distinctly acid reaction in the small intestine.

THE CLINICAL VALUE OF THE LACTOBACILLUS.

The widespread use, in digestive disorders, of milk that has undergone lactic acid fermentation is based on empirical clinical observations and on the belief that these products in some way better the bacterial conditions in the intestinal tract. Within the past year extravagant claims have been made in respect to the beneficial results to be expected in digestive disorders from the use of milk fermented with Metchnikoff's lactobacillus[13] or from the use of pills made from this lactic-acid-forming bacillus. I have for many years used fermented milks in the treatment of putrefactive disorders and have latterly made a number of experiments with milk fermented with Metchnikoff's lactobacillus. While I have not yet a sufficient number of observations on human beings to justify me in making a specific criticism of the lactobacillus milk, there are some features

12. I showed in 1897 that the injection of cultures of lactic-acid-forming bacilli into the intestinal tract had the effect of diminishing intestinal putrefaction in dogs. I was at this time unaware that Metchnikoff was making studies to determine the effect of certain lactic acid bacilli on human intestinal putrefaction. (On Certain Relations Between Bacterial Activity in the Intestine and the Indices of the Urine, Brit. Med. Jour., 1897, ii, p. 1847.)

13. This organism occurs as a long, straight rod, with square ends with a tendency to form threads. It is non-motile, aërobic, and grows well in the lower part of the tube. The organism is Gram-positive. Dr. Collins was unable to obtain growths on plain nutrient agar, on bouillon, on glucose agar, on glucose-bouillon, lactose bouillon or on saccharose-bouillon. Fairly good growths were obtained from bierwort-agar (10 per cent.). The organism grows well in milk, forming a soft, uniform curd, during the first thirty-six hours. The period required to bring about coagulation varies. The fermented milk is markedly acid. Later the coagulum becomes firm, and still later, undergoes liquefaction. The organism must be frequently transferred to retain its viability.

attending the use of the preparation which emerge clearly from the experiments already made and which I wish to mention here for the reason that they may serve the purpose of averting some therapeutic disappointments based on misconceptions regarding the theory of the action of lactic-acid bacilli in putrefactive intestinal disorders.[14]

The lactobacillus is an exceptionally active producer of lactic acid, and milk that has been fermented by it for two days at body temperature is so strongly acid as to render it repellant to many persons. A shorter period in the incubator yields a product that contains less acid and is more palatable. If milk thus fermented be fed to a dog in which the ileum passes directly into the sigmoid flexure, the feces will be found to have a slightly acid reaction and to possess the peculiar glutinous consistence characteristic of the milk itself. Moreover, by suitable cultural procedures the large, dominant, Gram-positive bacilli found in the intestinal contents in large numbers can be shown to be the lactobacilli of the milk.[15] These bacilli have, therefore, not merely preserved their morphology in the passage through the tract, but at least some of them are still viable. Moreover, if the ethereal sulphates of the urine be studied in dogs thus fed, as was done by Dr. Baldwin at my request, it will be found that the sulphates are excreted in very small amounts. I do not consider it proven that the ethereal sulphates are lower in normal dogs fed on lactobacillus milk than in dogs fed on reasonably clean cow's milk, but the figures obtained in my laboratory under these two conditions point to a slight difference in favor of the fermented milk.

It should next be noted that if one adds a pure culture of *B. aerogenes capsulatus* to a growing culture of the lactobacillus in milk, the former organisms make little or no progress in growth, perhaps because the milk is too acid, but more likely (in the cases at least where the milk has not attained the maximal acidity) because suitable anaërobic conditions and suitable food are wanting. On the other hand, if the *B. aerogenes capsulatus*

14. One claim made for the lactobacillus sold in New York is that it is superior to other fermented milks in being free from yeasts. Dr. Collins has found a yeast in some samples of this milk, and Dr. Pifford called my attention to the frequent occurrence of a difficultly cultivable yeast organism.

15. This was first shown by Dr. Catherine Collins in my laboratory.

be inoculated into fermentation-tubes containing litmus milk, it will produce gas and butyric acid there despite the presence of the lactobacillus.

Even when both types of organism were simultaneously inoculated it appeared that the lactobacillus was much impeded in its development. Finally, if a neutral reaction be maintained in a nutrient medium in which *B. aerogenes capsulatus* is growing, the lactobacillus is unable to make any progress. On the whole, we may say that the lactobacillus requires for its active multiplication much more special nutritive conditions than the *B. aerogenes capsulatus,* although the latter is at a disadvantage in needing anaërobic conditions, while the former prospers with or without oxygen, if grown on milk that has been sterilized and is permitted to contain some free acid.

We may now roughly picture what happens when the lactobacilli are introduced in large numbers into the digestive tract of a patient suffering from severe chronic saccharo-butyric putrefaction, with the *B. aerogenes capsulatus* as the dominant anaërobe to be controlled. We may assume, for the sake of argument, that in this patient the bacillus has succeeded in establishing itself at a level nearly as high as the middle of the small intestine, and that it makes its way to somewhat higher levels when an abundance of easily fermentable carbohydrate food reaches the former level. The introduction of a meal consisting wholly of milk fermented by the lactobacillus will provide conditions in the stomach and upper small intestine against which the gas-bacillus can probably make no progress, if one may judge from experimental conditions outside the body.

With the progressive absorption of the lactic acid formed by the lactobacillus the conditions would be altered in the direction of rendering them more favorable to the multiplication of the gas-bacilli located at or near the upper level of the infection, and the presence of a little unchanged sugar would, indeed, favor their growth. With the reduction of the acidity to a point approaching neutrality to litmus, the gas-bacilli would find opportunity for free growth on the casein of the milk provided a slight amount of sugar were present. The multiplication of the gas-bacilli would proceed with great rapidity as soon as the food and the intestinal juices became neutral or alkaline.

But just at this point we lack definite knowledge of the conditions that actually exist in the human intestine in such cases as we are picturing. Yet on one point a positive statement may be made with confidence. It is that intestinal putrefaction may continue very excessive in spite of the free or even exclusive use of milk fermented by the lactobacillus. There is no evidence whatever that the organisms of the lactobacillus milk or other fermented milks are able to restrain specifically the growth of *B. aerogenes capsulatus.* The intestine continues to hold these anaërobes in large numbers and in a living state, as can easily be shown in some instances by injecting into experimental animals intestinal material heated at 80 C. Such limitation of the anaërobes as is effected depends, I believe, on the absence of carbohydrates in the fermentable milk and on the presence of lactic acid. It is extremely doubtful if the lactobacillus multiplies on other food than the milk in which it is taken. The pathologic nature of some of these severer cases of infection through anaërobes will be discussed under another heading.

There can be no doubt that lactobacillus milk possesses certain advantages over natural milk in the treatment of chronic saccharo-butyric putrefaction. Of these, the chief are the ability of the bacilli to make lactic acid freely from soluble carbohydrates and to subdivide and transform the casein in such a way as to render it more readily absorbed than the casein of unchanged milk. Whether the lactobacillus by virtue of its powerful lactic acid-making properties possesses real advantages over the micro-organic ferments used in the production of kumyss, kefir, zoolak, etc., I shall not attempt to discuss here.

INFLUENCE OF EPITHELIAL ATROPHY.

It is a little singular that in the study of the factors entering into chronic gastroenteric disease so little stress has been laid on the influence of atrophy of the intestinal epithelium. There are, indeed, papers relating to epithelial atrophy in the small intestine in pernicious anemia, in the marantic atrophy of infants and in some other pathologic states, but I am not acquainted with any writings that indicate the significant consequences to the organism that may be expected to arise from extensive atrophies of the intestinal epithelium.

There are certain obvious difficulties in pursuing this subject from the standpoint of pathologic anatomy—especially the difficulty in obtaining autopsies at so early a period after death as to insure certainty that the epithelial denudation observed is not a postmortem change, and the difficulty in deciding on anatomic evidence whether the atrophies noted have been secondary to their associated pathologic states or have stood in a causal relation to such associated states. What I have to say in this connection is advanced as a suggestion directed toward further inquiries and is based on clinical observations and physiologic considerations rather than on experience with postmortem appearances.

There are at least three well-recognized conditions of disease in which the tongue shows atrophic alterations in its epithelium:

1. The glossitis known as wandering rash of the tongue, geographical tongue, or Müller's superficial glossitis.

2. The more general glossitis with epithelial denudation that occurs in the form of tropical diarrhea known as sprue.

3. The glossitis with denudation of epithelium that is so frequently noticed in persons with pernicious anemia.

Overlapping these conditions to an extent at present not accurately definable is the process of chronic saccharo-butyric putrefaction in which I have now observed epithelial atrophy of the tongue, of varying extent in several patients. That the saccharo-butyric process is highly developed in many persons suffering from pernicious anemia may be regarded as established. The process is also well developed in some persons, both children and adults, suffering from Müller's superficial glossitis, but I have not had sufficient experience to say how frequent is this association. In the case of indubitable sprue, I have had no opportunity for study. I have, however, had under observation an elderly gentleman with a chronic diarrhea due to saccharo-butyric putrefaction (from infection with *B. aerogenes capsulatus*) in which all the leading symptoms mentioned by Sir Patrick Manson in his description of sprue were present. It seems, therefore, well worth while to study sprue with the putrefactive anaërobes in mind and also

to inquire whether essentially the same condition does not sometimes occur in temperate climates.

In cases of wandering rash of the tongue[16] and in chronic saccharo-butyric putrefaction with and without advanced anemia, it is very common to find an entire absence of free hydrochloric acid in the gastric juices, and there is some reason to believe that where this is of years' standing it is apt to be associated with a degree of atrophy of the epithelium of the gastric tubules.

It is also certain that in many cases of pernicious anemia and in severe saccharo-butyric putrefaction generally the feces contain an excess of epithelial elements and mucus. As already stated, the epithelial structures may be strikingly abundant. It is clear that from this excessive desquamation no inference can be safely made as to whether atrophy of the epithelial structures of the intestine exists or not, the reparative ability of these structures of the mucous membrane of the intestine being of the highest order. There is, however, no doubt that after years of damage these cells lose in a measure their power of reproducing the epithelial structures of the gut.

Unfortunately we have at present no certain criterion for gauging the degree and extent of this damage to the epithelial structures. In my judgment, it is of the first importance for the physician to be able to form an estimate of the permanent damage that has been done to the epithelium, since the ultimate prognosis in cases of chronic saccharo-butyric putrefaction must be largely conditioned on the state of these epithelial cells. There are probably instances of epithelial denudation or atrophy in which regeneration is prevented by the continual presence of irritants formed in the course of the putrefactive decomposition induced by putrefactive anaërobes (doubtless sometimes in association with other bacteria, especially streptococcal forms). These are the instances in which suitable diet, lavage, rest, etc., are beneficial.

16. The epithelial atrophy in these cases may involve patches of mucous membrane of the mouth as well as the tongue. I consider it significant that the lesions of the tongue in Müller's superficial glossitis are certain to be temporarily greatly exaggerated by whatever conditions give rise to gastritis or gastroenteritis or to exacerbations of chronic gastroenteritis. We have in this fact an example of a perfectly definite relation between the state of the gastroenteric mucous membrane and the condition of the tongue. This relationship holds good even where it is possible to exclude food irritants as factors in the exacerbation of the lesions in the mouth.

On the other hand, it appears reasonable to suppose that there are patients in whom the epithelial atrophy has progressed so far that no therapeutic measures can avail to restore the epithelium to a degree of functional activity sufficient to maintain the organism, as a whole, on a level that makes it possible for the patient to escape being bed-ridden. If this conception of the pathologic basis of severe cases of chronic saccharo-butyric putrefaction be correct, it is unreasonable to condemn therapeutic measures which, though helpful in less advanced cases, are not effective in these extreme ones. What is required is more discrimination in the study of these cases and a better conception of what may naturally be expected from therapeutic interference.

The physiologic conditions present in extreme atrophy of the epithelium of the small intestine present a close parallelism to those in chronic nephritis with small granular kidneys. In both cases there is failure in function because of damage to epithelial structures. In the case of kidney disease this is obvious because the epithelial cells are closely concentrated in an organ presenting gross and microscopic appearances of a striking character. In the case of the intestine the significance of the lesions is overlooked because the damage is spread over a longer area of mucous membrane, the epithelial structures of which should, in reality, be regarded as constituting an organ despite the fact that they are not bound together by connective tissues into a structure so definitely limited as to impress obtrusively the eye of the examiner.

Another reason, perhaps, which has favored the underestimation of the importance of the epithelial atrophies in the intestine is the fact that one is apt to consider processes in the gut as going on within the lumen of the gut—and hence easily to be modified—rather than in the walls of the intestine itself. We have long been familiar with the conception that epithelial atrophy of the kidney may reach a point where it is unreasonable to expect to avert uremia. It is important for us to grow familiar with the analogous conception that ordinary therapeutic measures can not be expected to rescue from failing nutrition those patients who have acquired extensive intestinal epithelial atrophies. I am willing, however, to venture the opinion that we have

not yet learned to give such patients the fullest benefit of the cellular activities still possessed by them.

RESULTS OF EPITHELIAL ATROPHY.

In conclusion, I desire to sketch some of the possible consequences of extensive epithelial atrophy in the small intestine. Among such consequences one might expect an impaired secretion of the succus entericus, a diminished production of erepsin, a diminished secretion of secretin (the latter leading to diminished pancreatic activity). An impaired secretion of fluids from the blood into the intestine, as under the influence of saline cathartics, is to be expected under these conditions, and it is possible that we have here an explanation of the failure of saline cathartics to act readily in long-standing cases of intestinal inflammation. With impaired secretion must necessarily be coupled an impaired absorption of digestive products.

One may reasonably picture, moreover, a diminished capacity of the epithelial cells for decomposing certain cleavage products of digestion, as, for example, the amino acids which are known to be disamidized in the intestinal walls. Likewise the synthesis of fatty acids and glycerin into neutral fats might be expected to be less efficient than normal. Again one would expect an impaired power of binding certain toxic bodies formed in the intestine, as, for example, indol, which I have found to be bound with avidity by epithelial cells of the intestine. The lessened ability to transform toxic substances into less harmful ones may perhaps facilitate damage to red blood cells exposed to such poisons in the congested areas of the intestinal tract which are known to exist in some cases of disease from chronic anaërobic infections.

An increased permeability of the intestinal wall to bacteria is almost certainly a consequence of denudation of the epithelium. At present, however, it is impossible to form an opinion as to the extent to which this factor is operative in leading to injurious consequences secondary to disease of the digestive tract due to anaërobic organisms.

In chronic saccharo-butyric putrefaction we find at least some of the conditions which may be expected where there is reduced epithelial activity, especially a

permanently diminished power of resorption for proteids and fats and a consequent failure in nutrition.

CONCLUSIONS TO BE DRAWN.

Certain practical conclusions may safely be drawn from the facts advanced in the foregoing pages, and of these the chief are as follows:

In well-defined cases of saccharo-butyric putrefaction carbohydrate food is vigorously attacked by the intestinal bacteria and must therefore be carefully restricted in all cases. Fats of low melting point (butter, beef-fat) are well tolerated in moderate quantities and should be rather freely used, to replace carbohydrate in part, provided no increase in intestinal putrefaction is attributable to their use. Of proteins, those of milk and of beef are well tolerated and utilized in moderate amounts, and when taken with only small quantities of carbohydrates, but if eaten in abundance are followed by increased putrefaction, which is the more pronounced the more abundant are the associated carbohydrates.

In long-standing cases, with indications of epithelial atrophy, the restriction of all classes of foods may be essential to diminish putrefactive intestinal decomposition within limits that are essential to repress various obstructive symptoms. In order that the caloric needs of the body should not be too glaringly disproportionate to the food supply that can be tolerated these needs must be cut down by causing the patient for a time to rest in bed and to relinquish active mental occupation. This course usually leads to an increased tolerance and utilization of food-stuffs. During this period of rest the diet should at first consist mainly of proteids and fats (milk, fermented milk, minced beef, gelatin), the carbohydrates with low sugar content being very cautiously added as the signs of excessive putrefaction recede. In a certain number of patients such a period of rest and dietetic caution, lasting from two weeks to two months, is followed by great improvement. On the other hand, in patients with extreme epithelial atrophies and extreme, persistent putrefaction, little permanent benefit is to be expected from any method of treatment at present known, especially if nutrition has suffered severely and blood destruction has long been out of proportion to the powers of regeneration; and this conclusion holds especially true of patients in whom systematic rest and appropriate diet have already been conscientiously tried.

INDOLACETURIA

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ONE HUNDRED AND THREE DEARBORN AVENUE.
CHICAGO.

INDOLACETURIA

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In the course of investigations made on the urine of a child suffering from a chronic intestinal bacterial infection, it was noticed that the addition of concentrated hydrochloric acid to the urine led to the development of an intense rose color. The study of this color reaction showed it to be identical with the reaction described in 1882 by Nencki and Sieber as the urorosein reaction.[1] Although there has been considerable discussion as to the nature of this reaction, its exact nature has, up to the present, been left in obscurity. In the course of my study of the patient just mentioned it was determined that the mother substance or chromogen from which the urorosein is derived is a definite substance arising in the intestinal tract from the breakdown of tryptophan by bacteria. This substance is indolacetic acid. Further study has shown that the occurrence of indolacetic acid in the urine is not a rare occurrence and that it is usually associated with pathologic conditions of the intestinal tract. For this reason it has appeared to me worth while to bring together here such facts as have now come to light.

I have ventured to designate by the term indolaceturia the presence of indolacetic acid in the urine. The term is somewhat awkward and perhaps in other respects not without objection, but nevertheless I think it advantageous to make use of this term, as otherwise one is constantly confronted with the inconvenience of designating the condition by circumlocution.

It is desirable to sketch at the outset the characteristics of a urine containing indolacetic acid, in appreciable quantity. If to such a urine there be added an equal volume of concentrated hydrochloric acid, one of

1. Jour. f. prakt. Chem., 1882, xxvi, 333.

two results may be looked for: either the urine develops promptly a fine rose-red color which deepens in intensity during the first few minutes, or there is no development whatever of the rose-red color. In making this statement it is assumed for the moment that the urine does not contain indoxyl potassium sulphate in sufficient concentration to give rise to the occurrence of an appreciable color reaction from the presence of indigo. Urines occur which, on addition of concentrated hydrochloric acid, do yield indigo in sufficient amount to distinctly obscure the rose-red reaction, and it is necessary in such cases to resort to a special procedure in order to make the distinction certain between the presence of indolacetic acid and an indoxyl compound. The explanation of the different behavior of urines containing indolacetic acid toward concentrated hydrochloric acid is simple. If we make a watery solution of indolacetic acid or one of its salts and add to it concentrated hydrochloric acid, there is no change in color; but if we now add to the mixture some suitable oxidizing agent, as for example, one or two drops of a 0.1 per cent. solution of potassium nitrite, there is instantly developed the characteristic rose-red color. Experimental study has shown that those urines which yield the typical indolacetic acid color reaction on the addition of concentrated hydrochloric acid contain traces of nitrites. These nitrites are formed through the action of nitrifying bacteria in the urine,[2] possibly from the breakdown of urea, but more probably from the action of these bacteria on compounds of ammonia normally present in every urine. Since a mere trace of the nitrite is all that is necessary, in the presence of hydrochloric acid, to give rise to the rose-red or urorosein color, it may happen that the urine is not greatly clouded by the presence of nitrifying bacteria. It is noteworthy that the quite fresh urines from persons showing the urorosein reaction will not develop the color reaction without the addition of nitrites, whereas the same urines on standing in the laboratory for twelve or twenty-four or forty-eight hours will frequently give rise to the reaction, without the addition of a nitrite.[3]

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2. The relation of Nitrifying Bacteria to the Urorosein Reaction of Nencki and Sieber. Jour. Biol. Chem., 1908, iv, 239.

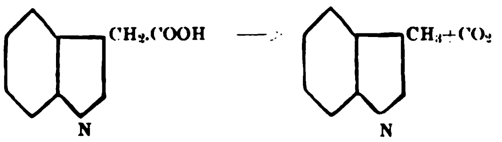
3. There are occasional exceptions to this rule, due to the fact that the urine may contain a trace of a nitrite when freshly voided.

It is possible that other oxidizing agents than nitrites at times play a part in the production of the color reaction. At present I know of no substance occurring naturally in the urine which will take the place of the nitrites in oxidizing the indolacetic acid chromogen with the typical resultant color reaction. The chief practical interest of the fact that nitrites are able to oxidize the indolacetic acid so as to yield the typical rose-red color lies in the circumstance that one may wholly overlook the presence of a considerable and significant amount of indolacetic acid in the urine simply by failing to add the necessary trace of a nitrite to the urine to be tested. Observers who in the past have found the urorosein reaction to be present in the urine have generally detected it simply by the addition of concentrated hydrochloric acid. In other words, the nitrites necessary for oxidation have happened to exist in the urine. I have found the indolacetic acid reaction many times present in urines which failed to give the reaction on the addition of hydrochloric acid alone, and its presence has been detected simply through the use of sodium nitrite or potassium nitrite.

It is worth while to consider briefly the chief properties of indolacetic acid as contrasted with the properties of the urorosein colored substance resulting from the oxidation of indolacetic acid through the action of hydrochloric acid and nitrous acids.

PROPERTIES OF INDOLACETIC ACID.

Well-purified indolacetic acid crystallizes from benzol as small, flat crystals with a melting point of 164° C. When heated a few degrees above the melting point it breaks down into carbon dioxid and skatol, a property of much value for the identification of the substance. This occurrence is indicated in the following equation:



Indolacetic Acid. Skatol.

Indolacetic acid is very slightly soluble in cold water, easily soluble in alcohol and in ether, less easily soluble in chloroform and only slightly soluble in benzol. A

solution of indolacetic acid when acidified with hydrochloric acid develops a violet color on heating in the presence of a very small quantity of ferric chlorid. A solution of indolacetic acid of the strength of 1:10000 behaves in this way. This reaction was first described by Salkowski,[4] who regarded it as especially satisfactory for the recognition of indolacetic acid or, as it was formerly called, skatol-carboxylic acid. If to a watery solution of indolacetic acid (1:1000) there be added a few drops of nitric acid (specific gravity 1.2) and a few drops of a 1 per cent. solution of potassium nitrite be added, the solution develops a cherry or rose-red color, according to the concentration of the indolacetic acid. The coloring matter thus developed may be sufficiently abundant to separate out. It is the coloring matter known to Nencki and Sieber as urorosein. The gradual development of a purple-red may be obtained by adding to the watery solution of indolacetic acid (1:1000) an equal volume of hydrochloric acid (specific gravity 1.2), to which is then added a few drops of a 1 per cent. solution of chlorid of lime. If instead of using chlorid of lime one employs a 1/10 per cent. solution of potassium nitrite, there is a gradual development of the urorosein color, and this method is the most satisfactory for detecting the presence of indolacetic acid in the urine. A solution of indolacetic acid, when boiled with a solution of paradimethylamidobenzaldehyd (Ehrlich's aldehyd), dissolved in hydrochloric or sulphuric acid, gives rise to a red color differing from the color obtained by the action of this reagent on indol in being both far less sensitive and in presenting a less purplish tinge. On boiling a watery solution of indolacetic acid with Millon's reagent, a yellow color is obtained.

PROPERTIES OF THE UROROSEIN COLORING MATTER.

The urorosein coloring matter as obtained by the action of strong hydrochloric acid and potassium nitrite on indolacetic acid is a rose-red or cherry colored substance which has the property of passing readily into amyl alcohol. It passes somewhat less readily into propyl alcohol, but can be satisfactorily extracted, especially if salted out. The spectroscopic examination of the red amyl alcohol solution shows the presence of a

4. Ueber das Verhalten der Skatolcarbonsaüre im Organismus. Ztschr. f. physiol. Chem., 1885, ix, 23.

characteristic absorption band in the green portion of the spectrum, its location being somewhat nearer the sodium line D than the line E. On treating the urorosein coloring matter with zinc and hydrochloric acid, it is readily reduced to a colorless substance, but through a cautious use of oxidizing agents the original color may be partially restored. The chemical constitution of the urorosein coloring matter has not been established. It is possible that it is a nitroso-indolacetic acid analogous to the nitroso-indol formed by the action of nitrites on indol. As, however, a similar and possibly identical coloring matter may be obtained through careful oxidation of indolacetic acid by means of other oxidizing agents than nitrous acid, it is not clear that we are justified without further study in regarding the urorosein reaction as due to the formation of nitroso-indolacetic acid.

METHODS OF TESTING URINE FOR THE PRESENCE OF INDOLACETIC ACID.

When the urine contains a moderate quantity of indolacetic acid—say 0.001 to 0.01 of 1 per cent.—the addition of an equal volume of concentrated hydrochloric acid and the careful addition of a few drops of a 0.2 per cent. solution of potassium nitrite suffices to bring out the urorosein reaction in an unmistakable manner. When the quantity is smaller or where other coloring matters exist in the urine, it may be less easy to establish the presence of indolacetic acid. Whenever any doubt exists as to the nature of the reaction suspected to depend on indolacetic acid, the following procedure should be employed: One hundred cubic centimeters of the urine should be evaporated to a volume of 20 c.c. After cooling, this concentrated urine should be carefully filtered. The filtrate is acidified with 1 c.c. of a 10 per cent. solution of phosphoric acid. This acidified and concentrated urine is now placed in a separatory funnel and extracted with from 40 to 50 c.c. of ether. It is not necessary to shake the funnel for more than five minutes, as any indolacetic acid that may be present will promptly pass in large part into the ether. After allowing the contents of the funnel to stand until a good separation takes place between the urine and the ether, the ethereal extract is drawn off and evaporated on the water bath. The residue is taken

up in 5 c.c. of water, and to this solution is now applied the test with concentrated hydrochloric acid and potassium nitrite. It frequently happens that urines which show only a moderate and obscure urorosein reaction, when the test is applied directly to them, yield an ethereal extract which gives a beautiful and typical reaction. With some urines there is difficulty in making a clean separation of the ethereal layer, owing to the formation of an emulsion, and in such cases a good separation can be brought about by the use of the centrifuge.

Urines which contain an abundance of indican develop enough indigo on oxidation with a nitrite to obscure the reaction dependent on indolacetic acid. The indigo that is separated may advantageously be removed from the solution by shaking with chloroform. The super-natant fluid then shows the characteristic rose color due to the oxidation of the indolacetic acid and, after decanting this colored urine, the color may be shaken out into amyl alcohol. Indigo red, as well as indigo blue, passes over into chloroform if the former happens to be present with the latter, and in all cases in which indoxyl derivatives are present in the urine it is best to concentrate the latter and extract with ether as above described.

It is necessary to use some care in employing the nitrites for the development of the urorosein reaction. Even urines which contain no indoxyl potassium sulphate (if one may judge by the inability to obtain indigo by means of the ordinary reagents, such as Obermeyer's) may develop a purple or violet color in place of the typical rose-red color. This variation in color from the typical rose-red has been found in some instances to be due wholly to the mode of oxidation with potassium nitrite. This is very clearly indicated when, through the cautious addition of nitrite, the typical rose-red color is developed in the upper portion of the test tube, whereas the violet color appears in the lower part where oxidation is less active, all transitional tints being observed between these two levels. As already stated, it is preferable to add the nitrites in very dilute solutions after strong hydrochloric acid has been added to the urine (or the extract of the urine) instead of previous to the addition of the hydrochloric acid.

I have observed a few instances in which the mere

addition of concentrated hydrochloric acid to the urine has been followed by the appearance of an intense greenish-blue or blue color, due to the presence of indigo. This phenomenon has been observed by me only in urines obtained from children suffering from severe gastroenteritis, although I have occasionally seen slighter manifestations of the same phenomenon in the urines of adults. In the cases in which this development of indigo has occurred, the excess of hydrochloric acid employed has always been great. In such cases the indigo must be shaken out with chloroform before the addition of the nitrite or, preferably, the urine should be extracted with ether.

The quantitative estimation of indolacetic acid can only be made with considerable difficulty, and I shall not here discuss the technic to be employed in the endeavor to recover the greater part of the indolacetic acid present in the urine. The greater part may, however, be recovered by suitable methods of extraction. For practical purposes it suffices to dilute the urine in any case of marked indolaceturia to a point at which it no longer gives a trace of urorosein on the addition of hydrochloric acid and potassium nitrite. The dilution required to cause a disappearance of the reaction can then be compared with the dilution at which an indolacetic acid solution of known strength ceases to give the reaction. It has been found by experiment that indolacetic acid in a solution holding one part of the acid in one hundred thousand parts of water still gives a faint color reaction. The highest estimated percentage of indolacetic acid in any urine yet observed by us has been 0.05, or 0.5 gram to the liter.

CLINICAL STATES ASSOCIATED WITH THE PRESENCE OF INDOLACETURIA.

A thorough study of the clinical states associated with indolaceturia has not yet been made. The systematic search for the presence of indolacetic acid in the urine through the use of strong hydrochloric acid and potassium nitrite will certainly bring to light many instances of indolaceturia which would be overlooked where strong hydrochloric acid is alone employed. The most pronounced examples of indolaceturia that have come to my notice have been in urines from patients with diabetes, typhoid fever or chronic enteritis. I have observed a strong reaction for indolacetic acid in

some cases of jaundice. Rosin found the urorosein reaction to be strong in many cases of pulmonary phthisis, and Garrod observed it in the urine of chlorotic patients. I believe that small quantities of indolacetic acid in the urine are common among chronic disorders of digestion. Various observers have noticed the occurrence of the urorosein reaction in osteomalacia, nephritis, carcinoma, ulcer of the stomach and perityphlitis. Dr. Baldwin has noted a strong reaction in the urine of a patient suffering from the vomiting of pregnancy.

For reasons which will appear in connection with the discussion of the pathologic significance of indolaceturia it is clearly desirable that in routine examinations of the urine, the tests for indolacetic acid should be applied. It is also desirable that a record should be kept of the relationship existing between the intensity of the reaction for indolacetic acid and the intensity of the reaction for indigo blue.

THE PHYSIOLOGIC AND PATHOLOGIC SIGNIFICANCE OF INDOLACETURIA.

An insight into the physiologic and pathologic significance of indolaceturia has been made possible by the researches of Hopkins and Cole[5] and of Ellinger[6] on the constitution of tryptophan. Tryptophan, which may with a high degree of probability be considered as indolamino-propionic acid rather than as skatolamino-acetic acid, is set free early in tryptic digestion of proteids. Normally, it is promptly absorbed from the intestine and either burned in the body with the formation of products not at present known, or appropriated in some synthetic process. Any obstacle or delay in the absorption of tryptophan favors decomposition by intestinal bacteria. *B. coli* certainly, and *B. bifidus* probably, are able to form indolacetic acid from tryptophan. It is certain also that many bacterial symbiotic combinations in the intestine are able to make indolacetic acid from tryptophan. It is noteworthy that indolacetic acid is liable to be absorbed as such when it has once been formed in the intestinal tract, and it is further noteworthy that this substance is relatively re-

5. The Constitution of Tryptophane and the Action of Bacteria on it, Jour. Physiol., 1903, xxix, 451.

6. Ueber die Constitution der indol Gruppe im Eiweiss (Synthese der sogen. Skatolcarbonsaüre) und die Quelle der Kynurensaüre, Ber. d. deutsch. chem. Gesellsch, May 4, 1906; No. 7, p. 1802.

sistant to the ordinary biologic processes of oxidation. Hence it is not surprising that the decomposition of tryptophan in the intestinal tract should be followed by the appearance of indolacetic acid in the urine. The acid probably appears in the urine as the salt of some common base and does not undergo any pairing with sulphuric acid or glycuronic acid. At present, at least, there is no evidence that such pairing occurs.

As both indol and indolacetic acid are derived from tryptophan and from tryptophan only, it is plain that there must be a reciprocal relation between the formation of indolacetic acid and of indol. Thus if the indol production from tryptophan be large, the opportunity for the production of indolacetic acid will be less than would otherwise be the case, and, *vice versa,* if indolacetic acid be formed in large amount, there is less opportunity for the production of indol, since there is at the present time no evidence that indol is ever derived from indolacetic acid. Only when the tryptophan available is abundant in the intestine as the result of delayed absorption can we expect to get both indol and indolacetic acid in abundance. Clinical experience is wholly in harmony with this view. When indolaceturia is most marked, indicanuria is not apt to reach the high grades of intensity sometimes observed when indolaceturia is absent. On the other hand, indicanuria of the most intense type is not apt to be associated with the highest degree of indolaceturia.

In one patient whom I have long had under observation, there was at one time a persistent and extreme indicanuria. Very slowly, as the patient showed improvement in the digestive trouble to which he was subject, there developed an intense indolaceturia which was long persistent and appeared to be at the expense of the indicanuria, since the latter gradually disappeared wholly. This behavior is intelligible if we assume that the breakdown can occur only through two paths, either (1) by way of indolacetic acid or (2) by way of indolpropionic acid. There are reasons for thinking that the latter is the source of indol, while it is extremely doubtful if indolacetic acid ever breaks down with the production of indol. We do not yet know under what conditions and through what stages of decomposition indolpropionic acid yields indol. It is clear, however, that indolpropionic acid is much less

stable than indolacetic acid and is more readily attacked by bacteria. I have made many experiments in order to see whether the bacterial breakdown of indolacetic acid will yield indol, and have never been successful in obtaining indol from this resistant substance, even when it has been exposed to the action of powerful indol producers. This fact strengthens me in the belief that indol is derived from indolpropionic acid and not from indolacetic acid.

Although there is no evidence that indolacetic acid yields indol through the action of bacteria, it is probable that under special conditions it does give rise to skatol. I have made numerous observations with culture media containing indolacetic acid, but no substance capable of yielding tryptophan, in the hope of obtaining skatol through the action of anaërobic skatol-producing bacteria. I have failed to obtain up to the present time any unequivocal evidence of the origin of skatol from indolacetic acid by means of bacteria. There remains, however, the important fact that through potash fusion and through the mere heating of indolacetic acid slightly above its melting point, this substance decomposes into skatol and carbon dioxid. For these reasons it is chemically reasonable that skatol should be derived from indolacetic acid.

It is thought by some investigators that the urorosein of the urine is identical with skatol-red, which is a coloring matter derivable by the addition of strong hydrochloric acid to the urine of animals or patients that have been fed on skatol. Thus Porchet and Hervieux[7] have recently maintained that urorosein and skatol-red are identical. They base this contention mainly on the fact that the skatol-red which appears after the administration of skatol to dogs gives the same typical spectroscopic band that is obtainable when the urorosein pigment is subjected to spectroscopic examination. I have elsewhere given in some detail the reasons which show that skatol-red and the urorosein reaction do not depend on the presence of the same mother substance in the urine.[8] Since discussing this subject I have been able to demonstrate the dependence of the urorosein reaction on the presence of indolacetic acid in the urine. When

7. Untersuchungen über das Skatol, Ztschr. f. physiol. Chem., 1905, xlv, 486.

8. Indolacetic Acid as the Chromogen of the "Urorosein" of the Urine, Jour. Biol. Chem., 1908, iv, 253.

this fact is realized by the writers who maintain that skatol-red and urorosein are identical it is evident that this contention will have to be abandoned.

The view that indolacetic acid arises from the decomposition of proteids in the intestinal tract is supported by the fact that I have been able to obtain from the intestinal contents of a patient showing a marked indolaceturia a substance showing the reactions for indolacetic acid. I have not yet, however, had the time to prepare this substance from the intestinal tract in sufficient quantity to make melting-point determinations and to demonstrate the formation of skatol on subjecting the substance to a temperature in the neighborhood of 180° C., these being the criteria which serve positively to identify indolacetic acid. It is entirely in harmony with the view that indolacetic acid arises from tryptophan decomposition in the intestinal tract that I have been able to induce a strong urorosein reaction in the urines of two healthy subjects who during several days ate a large excess of beef—from 3,000 to 4,000 grams daily. Previous to the eating of the beef in excess, the urines from these subjects failed to show the presence of indolacetic acid. My explanation of this induced indolaceturia is that in both instances the presence of such large quantities of proteid in the intestine gave rise to a delay in the absorption of tryptophan formed through digestive proteolysis and that in consequence of this delay indolacetic acid was formed through the action of bacteria on tryptophan. This observation leads me to suspect that there are instances of pathologic conditions in which the excessive use of proteins is at least a factor in the development of indolaceturia. I would refer in this connection particularly to two types of cases, namely, diabetes and certain instances of chronic intestinal indigestion in children. In diabetes there is a tendency for patients to restrict themselves in carbohydrates and to compensate for that restriction by increasing the fat and protein intake. The protein intake under these conditions may be 50 or 100 per cent. greater than would be the case in health. The same thing is true of a group of cases of chronic intestinal indigestion in children in which it is impossible to take more than very small quantities of carbohydrates without excessive and detrimental fermentation. These patients, moreover, are frequently unable to tolerate fats

in normal amounts. Under such conditions the physician tends to recommend a diet rich in protein.

It thus appears probable that the excessive feeding of proteins may be concerned with the development of indolaceturia. I do not maintain, however, that this is essentially the cause of the indolaceturia in the two classes of cases just mentioned. For I have seen the phenomenon in persons who are taking no excess of protein food. Moreover, in pathologic cases of indolaceturia, in which the protein food has been restricted, the indolaceturia may be still persistent. It is thus clear that, while the intake of a large amount of protein is a factor highly favorable to the development of indolaceturia, the occurrence of this condition must be regarded as depending rather on delayed absorption of tryptophan and suitable bacterial conditions than on mere overfeeding with proteins.

Assuming that the hypothesis set forth in this paper is correct and that both indolacetic acid and the indol formed in the intestinal tract are derived from tryptophan through the action of bacteria, the fact remains still to be accounted for that sometimes the decomposition of tryptophan yields mainly indolacetic acid and at other times mainly indol. I am at present unable to offer a satisfactory explanation for this selective breakdown of tryptophan. As is well known, bacteria of the *B. coli* group are capable of clearing tryptophan to indol and, as already mentioned, it was shown by Hopkins and Cole (an observation confirmed by Dr. Dakin and myself) that these organisms are able to form indolacetic acid from tryptophan. It is doubtless true that in the intestinal tract the bacteria associated with the colon bacilli play a part in determining the direction of the main cleavage of tryptophan, and I think it possible that through the careful study of the symbiotic action of bacteria on this substance it will be possible to gain an insight into the different bacterial conditions that determine the production of indolacetic acid rather than indol.

It is not my intention here to discuss the pathologic consequences of indolaceturia. I may point out the fact, however, that, since indolacetic acid is not paired in the organism like indol and skatol and phenol, it may have an opportunity to act directly on the nervous system when absorbed in considerable quantities. A com-

parison of the action of indolacetic acid, both on the muscle functions and the functions of the central nervous system with the effects produced by indol and skatol, is thus a distinct desideratum. Dr. Lee, who has interested himself in the effects of indol and skatol on muscle fatigue, has kindly offered to study the effects of indolacetic acid on the living muscular substances. I have not yet had sufficient experience in the therapeutic modification of indolaceturia to express any opinion as to the best method of ridding the organism of this condition, but in any scheme of treatment the diminution of protein food would have to be considered.

THE RELATION OF NITRIFYING BACTERIA TO THE UROROSEIN OF NENCKI AND SIEBER.

BY

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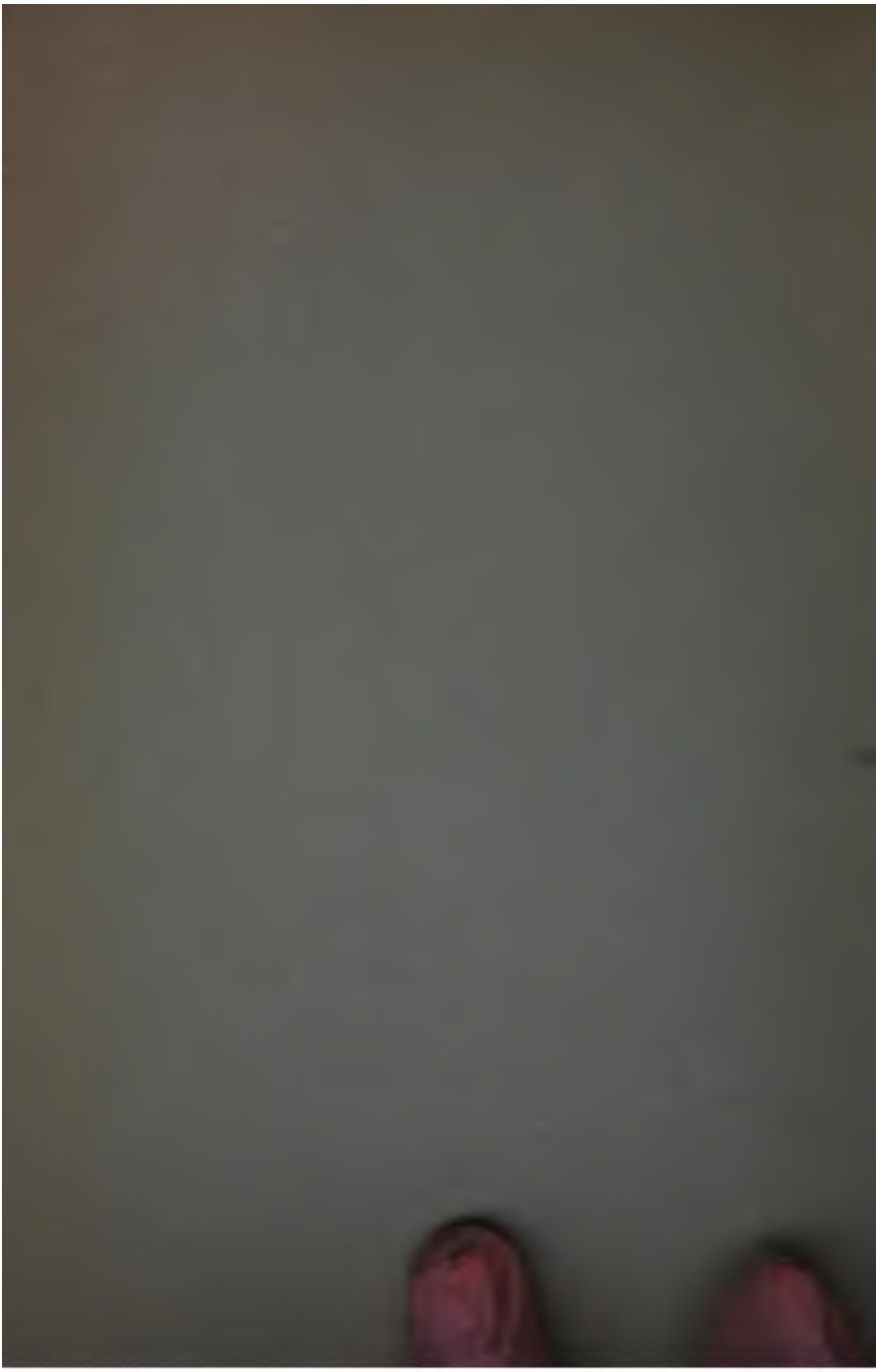

THE RELATION OF NITRIFYING BACTERIA TO THE UROROSEIN REACTION OF NENCKI AND SIEBER.

By C. A. HERTER.

(Received for publication January 7, 1908).

In 1892 Nencki and Sieber[1] found that when pure hydrochloric acid was added to the urine of a diabetic patient under their observation, there resulted a beautiful rose-red color. They were sufficiently interested in this little discovery to study the cause of the rose-red coloration, and although they did not succeed in obtaining in a chemically pure state the substance concerned in this reaction, they at least established its most important characteristics, thus rendering it easy for others to recognize its presence. In the course of their studies Nencki and Sieber noted the presence of urorosein (as they called their new coloring matter) in the course of a variety of diseases, such as osteomalacia, nephritis, typhoid fever, carcinoma of the œsophagus, ulcer of the stomach and perityphlitis. Although they obtained well-marked reactions for the first time from the urine of a diabetic patient, they failed to elicit it in some other instances of this disease. They state that the coloring-matter was found in about 10 per cent of the pathological urines examined by them. On the other hand, they failed to find it in the urine of healthy individuals.

The following are among the chief features noted by Nencki and Sieber in respect to their urorosein. The rose color was developed in the cold by the addition of sulphuric or hydrochloric acid. The coloring substance so induced was found to pass readily into amyl alcohol. The spectroscopic examination of the red amyl alcohol solution showed the presence of a characteristic absorption band in the green portion of the spectrum, its location being somewhat nearer the sodium line D than the line E. Only mineral acids were observed to bring out the reaction, acetic acid, for example, being unable to induce it.

[1] *Journ. f. prakt. Chem*, xxvi, p. 333, 1882.

Nencki and Sieber state that their urorosein differs distinctly from urobilin and indigo blue and they further maintain that it differs from coloring matters observed previously in pathological urines. They emphasize especially the fact that the red coloring matter described by Plosz[1] is wholly different from their urorosein. On the other hand they point out certain resemblances to the rosaniline dyes, especially to fuchsin, which in very dilute solutions not only gives the same nuance as urorosein but also gives a similar absorption band, but one somewhat nearer the violet. They observed also that commercial acid fuchsin in alcoholic solutions shows an absorption band, the position of which is exactly that of urorosein—a point to be noted in this connection as illustrating the fact that two dyes constitutionally different, even according to the view of Nencki and Sieber, may have the same spectrum.

At the conclusion of their paper Nencki and Sieber suggest the possibility that their urorosein arises from a mother substance formed in the intestinal canal as a decomposition product of protein through some form of bacterial organism of not very common occurrence. They suggest further that an aromatic base, isomeric aldehyde collidin, obtained through the decomposition of gelatin and proteid through putrefaction with pancreas, may arise during intestinal putrefaction and constitute the mother substance of urorosein.[2] Nencki and Sieber mention that Gautier, who also obtained this base from the decomposition of fish muscle, looked upon it as the ptomaine of Selmi. The authors also expressed confidence that direct feeding experiments with the ptomaines would show whether this view is correct or not. Evidently they failed to institute such experiments.

Finally it was concluded by Nencki and Sieber that in the substance called urorosein we have a new type of urinary coloring matter differing on the one hand from those coloring matters

[1] "Ueber einen neuen krystallinischen farbigen Harnbestandtheil." *Zeitschr. f. physiol. Chem.*, vi, p. 504, 1882.

[2] Aldehyde collidin fails to give any color reaction when treated with potassium nitrite in the presence of strong hydrochloric acid. It therefore cannot be the mother substance of urorosein, for reasons which will be made evident in this paper.

that are derived from bile pigment and on the other from those that have their origin in skatol and indol.

The most important recent work on urorosein has been done by Rosin,[1] who found that the pigment is present in small quantities in every normal urine. On this point Garrod and Hopkins differ from Rosin for they say that the coloring matter occurs only occasionally under normal conditions. Rosin observed the reaction to be especially active in persons suffering from pulmonary phthisis while Garrod[2] frequently found it in the urine of chlorotic patients. It is noteworthy that Rosin observed the coloring matter in greater quantities in persons on a vegetable diet than in those on a meat diet, and also found that the urine of horses contains considerable of the coloring matter, though less than that of cattle.[3] Rosin furthermore succeeded in crystallizing a chromogen of urorosein by precipitating the concentrated alcoholic solution (obtained by treatment with lead acetate) with ether. This chromogen occurred in colorless, transparent needles which readily dissolved in alcohol and water but not in ether or chloroform. It was imperfectly precipitated by lead acetate as a lead salt soluble in alcohol. The watery or alcoholic solutions as well as the crystals themselves develop a red color in contact with a mineral acid and an oxidizing agent. The pure substance on the addition of hydrochloric acid and barium chloride gives no separation of barium sulphate and can therefore not be regarded as an ethereal sulphate. According to Garrod and Hopkins the chromogen is largely thrown out by saturating the urine with ammonium sulphate—an observation which I can confirm. The alcoholic extract of the precipitate is made red by acid and then shows together with the urorosein band a weak urobilin absorption band. A separation of the chromogen is possible if one only adds enough ammonium sulphate to

[1] "Ueber das Indigoroth (Indirubin)" Virchow's *Archiv. f. path. Anat. u. Physiol.*, cxxiii, p. 519, 1891; "Ein Beitrag zur Lehre von den Harnfarbstoffen (Ueber das sogenannte Urorosein, Harnrosen)," *Deutsch. med. Wochenschr.*, xix, p. 519, 1893.

[2] Garrod and Hopkins. "On Urobilin. Part 1, The Unity of Urobilin" *Journ. of Physiol.*, xx, p. 112, 1896. Also Garrod: "The Spectroscopic Examination of Urine," *Edinburgh Med. Journ.* n. s., ii, p. 103, 1897.

[3] In observations on the urines of two horses I failed to obtain the reaction even on addition of nitrites.

the urine to cause cloudiness, this cloudiness being dependent on the chromogen itself and the urobilin remaining in solution.

I have lately had an opportunity to study a urine showing the urorosein reaction of Nencki and Sieber in a marked degree. This urine was obtained from a child seven years of age suffering from a peculiar variety of intestinal decomposition due to an abnormal type of intestinal flora. The child was very much retarded in skeletal and muscular development but was mentally alert, although undeveloped. A noteworthy peculiarity of the condition was marked protrusion of the abdomen, due presumably to long standing distension of the intestine with gases. This case was brought to my notice by Dr. L. E. Holt and is similar in type to a number of cases occurring in children which I have studied, though less carefully than the present case, in conjunction with Dr. Holt. I desire to report here certain observations upon the urorosein reaction as it was met with in this case, believing that the facts developed in the present instance are such as to place the urorosein reaction of Nencki and Sieber in a different light from that in which they regarded it.

It was observed that samples of the urine from the patient just mentioned, when treated with concentrated hydrochloric acid, frequently developed a brilliant and beautiful rose-red color which faded after some hours. It was found that the coloring matter thus developed corresponded in all essential particulars to the urorosein described by Nencki and Sieber, especially in respect to its solubilities, its behavior with reducing agents and oxidizing agents, and the position of the spectroscopic absorption band observed in amyl alcohol solutions. It was soon observed, however, that in one essential respect the color phenomenon observed in this urine did not coincide with the description of these authors. It was found that the urorosein reaction in our case developed only in such urines as had stood in the laboratory at least twelve or twenty-four hours. Fresh urine from the same patient under no circumstances gave the reaction. The onset of the reaction coincided with the development of a turbidity in the urine due to the presence of bacteria, and suggested a possible bacterial origin for the urorosein reaction. This view was confirmed by experiments directed to this point. It was found that if a fresh urine from our patient was inoculated with bacteria

from the urine which had already developed the urorosein phenomenon, the color reaction was obtainable in from twelve to twenty-four hours, the urine remaining in the laboratory during this period. On the other hand, the control urines which were carefully protected from contamination failed to develop the reaction.

The organisms responsible for this change in the urine were easily obtained in pure culture by plating on bierwort agar plates. They occurred as small, Gram-negative bacilli from 0.75 to 1.5μ in length and from 0.5 to 0.7μ in width. They were usually elliptical or ovoidal in shape, younger forms occurring as diplococco-bacilli or even as diplococci. Some of these coccal forms did not attain a diameter greater than 0.3μ. These organisms produced a moderate amount of gas in sugar bouillon. They did not coagulate milk. They possessed one characteristic which is of special importance in the present connection, namely, their capacity to form nitrites. It was found that sterile urines developed nitrites after inoculation with a pure culture of these bacteria. I have not had an opportunity to compare this nitrifying organism with recognized types of nitrifying bacteria.

In the course of conversation with Dr. Dakin regarding the urorosein phenomenon he suggested the possibility that the bacteria which were evidently concerned in the development of the urorosein reaction might be operative through their ability to form nitrites or nitrates. Experiments showed that this is indeed the case. It was observed that the urine of our patient did not develop the urorosein reaction until nitrites had been developed in it as the result of bacterial action. The presence of nitrites was easily shown by the test with metaphenylendiamin and also by the reaction of Tromssdorff, which depends on the liberation of hydriodic acid from potassium iodide in the presence of starch and zinc chloride, on the addition of dilute sulphuric acid to a urine which contains nitrites. In confirmation of the existence of such a relationship between the presence of nitrites and the development of the urorosein reaction, it was found that in fresh urines from our patient which gave absolutely no color reaction with strong hydrochloric acid, the addition of a few drops of a two-tenths per cent solution of sodium nitrite to the contents of the test-tube regularly and rapidly determined the development

ıroro in reaction which was in every respect typical. There o loubt that so far as our case is concerned the develop- the urorosein reaction is contingent upon the presence of ces in the urine.

was thought possible that the presence of nitrates might ert an effect on the development of the urorosein reaction ilar to that exerted in the case of the nitrites. It was found, however, that the urine was free from nitrates. Moreover, although it was found that by adding sodium nitrate to the urine there could be obtained evidence of the urorosein reaction when strong sulphuric acid was added, the color reaction was obtained with such difficulty and so imperfectly as to make it entirely clear that the presence of nitrates would not suffice to explain a reaction such as was regularly obtainable from the urine under consideration.

Although the urorosein reaction was so easily obtained from the fresh urine in our case through the addition of potassium nitrite, it was impossible to obtain the reaction, except as a very faint suggestion from any of the normal urines which were examined by a similar procedure. Evidently, therefore, two distinctive features contribute to determine the formation of the so-called urorosein—one, the liberation of nitrous acid, the other the presence of some chromogen peculiar to the urine in question, or at least existing in it in a far larger amount than in ordinary normal urines. The question of the nature of the urorosein reaction thus hinges on the understanding of the influence of each of these factors.

The relation of the nitrites to the development of Nencki and Sieber's reaction is apparently simple and depends on an oxidation of the mother substance or chromogen of the urorosein. There is no reason to think that the influence of the nitrite depends on the introduction of the nitroso group into the molecule of the mother substance, as in the case of the nitroso-indol reaction. The reason for believing this reaction to depend on simple oxidation is that a color closely resembling urorosein is obtainable through the oxidizing action of a number of different substances, as, for example, potassium permanganate, potassium nitrate, potassium persulphate and chloride of lime. In each case, however, there is a strong tendency to over-oxidation and in consequence of this the urorosein color never develops in full

degree throughout the test-tube, but is limited both in the extent of its appearance and in its duration. The oxidation of the mother pigment by nitrous acid is thus much to be preferred to that of other oxidizing agents, such as chlorine or nitric or sulphuric acids. But even with potassium nitrite some precautions must be taken to guard against over-oxidation on the one hand and under-oxidation on the other. Experience shows that it is best to add strong hydrochloric acid to the urine before adding the nitrite solution, the action of which can thus be carefully controlled. On the other hand, if the nitrites be added to the urine before the addition of strong hydrochloric acid, the maximal color reaction is liable not to be obtained owing either to excessive oxidation or to under-oxidation. In routine work it is probably best to employ an equal volume of strong hydrochloric acid in making this test, although frequently a very marked reaction may be brought out by using a smaller proportion of the acid.

It deserves to be noted that the urorosein color reaction does not always develop in a unifom manner. For example it was found that the urine from our patient sometimes gave with hydrochloric acid and potassium nitrite a purple or violet coloration rather than the typical rose-red color. It was at first thought that this violet color might be due to the formation of indigo, as it was otherwise difficult to explain. It was, however, noticed that this reaction could sometimes be obtained from a urine presumably containing no indoxyl-potassium-sulphate, if one may judge by the inability to obtain any evidence of indigo by means of the ordinary reagents, such as Obermeyer's. It was later observed in this connection that the variation in color between the typical rose-red and the violet depended wholly upon the mode of oxidation with potassium nitrite. This was very clearly shown in instances where through cautious addition of nitrite the typical rose-red color was developed in the upper portion of the test-tube, while the violet color appeared in the lower part, where oxidation was less active, all transitional tints being observed between these two levels. Finally, it may be said in this connection that the violet coloring matter just mentioned differs from indigo in not being removed by chloroform.

As regards the mother substance which constitutes the basis of the urorosein reaction, it is not possible to state its chemical

in their nuances are liable to show close similarity in regard to their spectroscopic bands, without this being actual evidence of identity. A good illustrative instance may be cited from the original paper of Nencki and Sieber in which they observed that acid fuchsin—a pararosanilin dye which gives a closely similar color to that of urorosein—also gives the same spectroscopic band. Yet Nencki and Sieber did not claim that urorosein and pararosanilin sulphonic acid are identical in chemical constitution because of the similarity of their spectroscopic behavior. Thus we may say in respect to the controversy over the relation between urorosein and skatol red that this must be settled on other evidence than the coincidence of the spectral bands from these two coloring matters. There are three features of difference between urorosein and skatol red which appear to me significant and even decisive in the discussion of this question. In the first place the two coloring matters are different in respect to their nuances and in regard to their solubilities. The rose tint of urorosein is lighter, brighter and more purplish than the red of skatol red. Urorosein is readily soluble in water but insoluble in ether and chloroform; skatol red is little soluble in water but somewhat soluble in ether and chloroform. In the second place, if one administers to a dog a dose of skatol, by mouth or subcutaneously, the urine will later contain skatol red, that is, a coloring matter which may be developed by the addition of strong hydrochloric acid to the urine. The urine does not, however, under these circumstances contain urorosein. I have not found it possible by the addition of strong hydrochloric acid and potassium nitrite (or any other oxidizing agent) to develop a coloring matter resembling urorosein in its nuance and its solubilities. Finally in the case which has formed the subject of the present study, I have been unable to obtain from the feces the slightest indication of the presence of skatol, notwithstanding that a large number of examinations have been made with a view to this end. The objection might be made to this argument that such skatol as had been formed in the intestine might have been absorbed from the upper part of the colon or from the small intestine and hence could not come within the range of observation. In reply to this, however, it may be said that on two occasions the contents of the bowel were largely and rapidly

evacuated and that even under these conditions not a trace of skatol was obtainable, although the usual intensity of the urorosein reaction remained apparently unchanged during the period corresponding to this cartharsis. At least, therefore, in the case under observation it may be claimed with fairness that the urorosein reaction has nothing whatever to do with the absorption of skatol from the intestinal tract. Nor do I think any serious contention can be made that skatol could have been formed in the cells during intermediary metabolism.

As regards a possible intestinal origin for the urorosein reaction it is necessary at present to speak with much caution. I have succeeded in obtaining through the action of the intestinal bacteria from the case under observation a coloring matter which appears to have the chief characteristics of urorosein. This coloring-matter was obtained by subjecting sterilized whole milk to the action of very large numbers of fecal bacteria, among which organisms of the bifidus type were extremely numerous. The bacterial process resulted in active fermentation and considerable solution of coagulated casein but no putrefactive products were present. When milk was similarly inoculated with small numbers of bacteria of the same origin there occurred coagulation of casein with little or no digestion and a not very active fermentative process. Under these conditions the urorosein reaction was not obtainable. In an experiment made with a view to inducing the presence of the urorosein mother substance in the urine, through filling the intestine with the fermented milk which gave the urorosein reaction, no success was obtained.

CONCLUSIONS.

1. The urorosein reaction of Nencki and Sieber sometimes and perhaps always depends on bacteria for its development where the reaction is induced by adding concentrated hydrochloric acid to the urine. From a urine giving this reaction a pure culture may be obtained which is capable of altering a sterile urine so that it in turn will give the typical reaction with hydrochloric acid.

2. The bacteria which assume this role are capable of forming nitrites in a previously sterilized urine and it can be shown

ui rosein reaction depends on the liberation of nitrous strong acid which is employed.

nitrites exert their influence on the chromogen as ially a propriate oxidizing agents and probably not through rmation of a nitroso compound. Doubtless the presence of urorosein chromogen would be frequently detected where it ow overlooked owing to a failure to employ nitrites in making the test.

4. Urorosein is distinct from skatol red and its chromogen occurs quite independently of the absorption of skatol from the intestinal tract.

5. The chemical constitution and physiological or pathological significance of the urorosein chromogen are at present unknown.[1]

Note on the Influence of Bacteria upon the Behavior of Urine Containing Indoxyl Potassium Sulphate toward Concentrated Hydrochloric Acid.

On account of the frequency with which observations are made on the indican of the urine it is worth while to record the following facts with respect to the influence of bacteria on the action of strong hydrochloric acid on urines containing indoxyl potassium sulphate. These facts were noted in the course of the preceding study on the urorosein reaction.

When bacteria concerned with acid fermentation were transferred from a urine giving the urorosein reaction to a series of urines from normal persons it was found (as stated above) that none of these urines developed the urorosein reaction. It was, however, observed that when concentrated hydrochloric acid was added to some of these urines they were found to develop a violet tint, whereas control samples that had not been inoculated failed to give this tint or else gave it in much slighter degree. On shaking with chloroform this took on the color of indigo. As this behavior was seen only in cases where the urine could be shown by Obermeyer's reagent to contain some indican, it is likely that the blue coloration of the chloroform is in reality due to indigo. I have not studied the phenomenon sufficiently to be certain as to the part played by the inoculated microörganisms. The presence of nitrites of bacterial origin may lead to a better oxidation of indican in the presence of strong hydrochloric acid than when the latter is alone employed. In some instances it was found that a stronger reaction for indigo could be obtained from a contaminated urine by merely adding to it strong hydrochloric acid than by the use of Obermeyer's reagent. For this reason it is evident that

[1] While this paper was going through the press I was able to obtain conclusive evidence that the urorosein chromogen is in reality indol acetic acid. I shall present this evidence in the next number of this journal.

erroneous conclusions may be reached as to the amount of indican present, if a urine has undergone the type of acid fermentation here observed. As is well known the intensity of the indican reaction given by a urine is usually decreased by alkaline decomposition. I have not before been aware that acid fermentation may lead to error in the opposite direction.

I have not examined the phenomenon closely enough to know whether other bacterial products than nitrites are ever responsible for the occurrences in question.

PRESS OF WILLIAMS & WILKINS COMPANY
BALTIMORE

ON INDOLACETIC ACID AS THE CHROMOGEN OF THE "UROROSEIN" OF THE URINE

BY

C. A. HERTER

FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
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ON INDOLACETIC ACID AS THE CHROMOGEN OF THE "UROROSEIN" OF THE URINE.

By C. A. HERTER.

(Received for publication, January 25, 1908.)

In the last number of this *Journal* it was stated that the mother substance or chromogen on which the urorosein reaction of Nencki and Sieber depends is still unknown. Since this paper was written I have had an opportunity to make experiments which show conclusively that the chromogen of the urorosein reaction is in reality indolacetic acid. Inasmuch as the nature of this color reaction of the urine has heretofore been involved in much obscurity, it is desirable to record at the present time the facts which show the relation of indolacetic acid to the urorosein reaction.

In the publication just mentioned, it was stated that it was possible to obtain by extraction of the pathological urine a solution free from color which reacted characteristically with hydrochloric acid and potassium nitrite and which further gave color reactions with Ehrlich's paradimethylamidobenzaldehyde and with Millon's reagent. Since then it has been found that not only in these respects but in more important ones the chromogen material contained in the urine extract corresponds in every essential particular to indolacetic acid. The opportunity to make this comparison I owe to the fact that Dr. Dakin was so obliging as to prepare for me a quantity of indolacetic acid. This indolacetic acid was obtained in crystalline form by the method first used by Hopkins and Cole.[1] These investigators showed that when tryptophan is acted upon by *B. coli* under partially aërobic conditions, indolacetic acid is formed in considerable quantity in addition to indol.

[1] Hopkins and Cole: "Contributions to the Chemistry of Proteids," pt. ii, "The Constitution of Tryptophane and the Action of Bacteria upon it," *Journ. of Physiol.*, xxix, p. 451, 1903.

On comparing the properties of solutions of crystalline indolacetic acid with the properties of the crystalline substance obtained from a pathological urine derived from a patient suffering from a peculiar type of intestinal bacterial decomposition, it was found that the two agree in the following respects.

(1) The colorless crystals obtained from the ethereal extract of the urine gave with concentrated hydrochloric acid and potassium nitrite a brilliant rose-red, possessing exactly the same nuance as that yielded by a solution of indolacetic acid. This color is very characteristic and differs distinctly from the color given by nitrosoindol.

(2) It was found that when the coloring matter prepared by the action of nitrous acid and strong hydrochloric acid was extracted in amyl alcohol it gave a spectroscopic absorption band indistinguishable from that yielded by the similarly prepared coloring matter from indolacetic acid. This absorption band is very sharply defined, at least on that edge lying toward the D line, and is located in the green portion of the spectrum.

(3) Both indolacetic acid and the crystalline substance recovered from the urine give the same red color reaction with paradimethylamidobenzaldehyde—a reaction differing from that obtained from indol in being much less sensitive as well as not identical in tint.

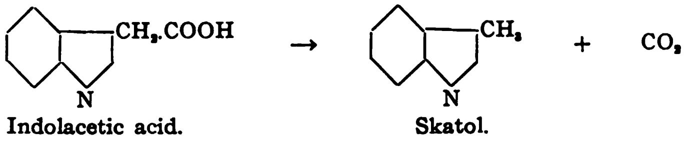
(4) Both substances give the same color reaction with Millon's reagent. As, however, this reagent contains nitrous acid, the significance of this reaction is probably the same as that described in (1). The color obtained is yellow rather than rose-red.

(5) Both substances give the same color reaction with ferric chloride and hydrochloric acid. This is a very delicate and characteristic color reaction which should be carried out with a few drops of a much diluted solution of ferric chloride. The cherry red color develops on heating. This reaction was first described by Salkowski[1] who regarded it as especially satisfactory for the recognition of indolacetic acid.

(6) The most important respect in which there is identity of chemical behavior between indolacetic acid and the substance isolated from the urine is the formation of skatol on heating. If

[1] Salkowski: "Ueber das Verhalten der Skatolcarbonsäure im Organismus" *Zeitschr. f. physiol. Chem.*, ix, p. 23, 1885.

indolacetic acid be heated to a point somewhat above its melting point (melting point, 163° to 164° C.), namely, to about 200° C. the acid loses carbon dioxide and is converted into skatol, in accordance with the following representation:



The operation is best carried out by immersing in a paraffin bath a long, narrow test-tube containing the substance to be tested. The skatol derived from the breakdown of the indolacetic acid condenses on the surface of the test-tube. The upper half of the test-tube is cut off from the lower half and introduced into a larger test-tube. The crystalline material adhering to its inner surface is now extracted with a few cubic centimeters of hot water. The solution thus obtained is tested with paradimethylamidobenzaldehyde in acid solution. On heating a solution containing skatol, to which a few drops of Ehrlich's aldehyde solution has been added, there develops a purple-blue color which deepens on the addition of concentrated hydrochloric acid. On cooling, the blue color gains in intensity. The blue color so obtained passes readily into chloroform. This characteristic reaction for skatol was obtained in its fully developed form from the crystalline substance extracted from the urine.

The foregoing features of agreement between our substance and indolacetic acid suffice to identify the former with the latter. In addition, however, it has been possible to obtain the substance from the urine in a well purified state (recrystallized from benzol) and in sufficient quantity to determine its melting-point, which proved to be 160° to 162° C. There is therefore essential agreement between the known properties of our substance and indolacetic acid, and I have no hesitancy in identifying the urorosein mother substance with indolacetic acid.

It is a little singular that the relationship between the urorosein of the urine and indolacetic acid should not have been earlier discovered. The publication of Nencki and Sieber in which they first described the urorosein reaction was made in 1882. In 1886

Salkowski[1] not only described the properties of indolacetic acid and its formation by bacteria during putrefaction, but also undertook a study of the behavior of the acid in the organism. He described indolacetic acid under the name of skatolcarboxylic acid. Salkowski made a series of observations upon the fate of indolacetic acid in the animal organism. He found that this substance, when administered to rabbits (by stomach) in moderate doses (e. g., 0.4 gm.) found its way to a considerable extent into the urine without undergoing change. He did not succeed in recovering the entire amount given, but this may have depended upon imperfections in the method. That the acid is not burned to any great extent in the organism was shown by the fact that after very moderate doses (e. g., 0.01 gm.) all the characteristic reactions for indolacetic acid could be obtained from the urine of the experimental animal. It would not have been remarkable for Salkowski to have suspected the relation of indolacetic acid to urorosein had he kept in mind the results published by Nencki, nor would it have been surprising had Nencki suspected the nature of his urorosein reaction after reading the observations of Salkowski upon indolacetic acid. Neither writer, however, appears to have concerned himself with the work of the other. Rosin, in 1893, stated that he obtained a small amount of crystalline substance from the urine of patients showing a urorosein reaction, which was capable of yielding certain typical color reactions. He failed however to bring his substance into relation with indolacetic acid.

In the patient from whom was obtained the urine which yielded indolacetic acid, experiment has made it clear that the intestinal tract contains bacteria which are capable of decomposing proteids with the production of indolacetic acid and there can be no doubt that the indolacetic acid of the urine is derived from absorption by the intestinal tract. That this substance is frequently formed to some extent in the course of intestinal putrefaction, was suspected by Salkowski, but was never demonstrated by him or by subsequent writers. I am able to state that the intestinal contents of the patient whom I have had an

[1] *Loc. cit.*; also "Zur Kenntniss der Eiweissfäulniss, II: Die Skatolcarbonsäure, nach gemeinschaftlich mit H. Salkowski in Münster i/W. angestellten Versuchen," *Zeitschr. f. physiol. Chem.*, p. 8.

opportunity to study regularly contains a small quantity of indolacetic acid, sometimes together with indol, sometimes without the latter, and never with skatol.

I hope in the near future to study the occurrence of indolacetic acid in the intestinal tract under pathological conditions[1] and to ascertain the nature of the bacteria which give rise to this decomposition. In the case of marked indolaceturia which I have had under observation one fact regarding the intestinal flora stands out with great clearness. This is the nearly entire absence of the ordinary putrefactive bacteria which lead to the saccharo-butyric form of intestinal putrefaction. The character of the bacteria actually present will be described in another communication. It may be stated here however that the dominant form, *B. bifidus communis* (Tissier) grown on glucose bouillon yielded a substance which gave the characteristic color reaction with potassium nitrite and hydrochloric acid.

In the light of what has been said in this paper it is evident that bacteriologists employing the nitrite reaction for indol should take great care not to confound indolacetic acid with indol. The test for indol should never be made except on the distillate.

[1] Under physiological conditions there may occur a degree of indolaceturia if the subject consumes very large quantities of meat. This fact will be amplified in a subsequent publication.

NOTE ON THE INFLUENCE OF MEAT ON THE DIMETHYLAMIDOBENZALDEHYDE (EHRLICH'S ALDEHYDE) REACTION OF THE URINE

BY

C. A. HERTER

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NOTE ON THE INFLUENCE OF MEAT ON THE DIMETHYL-AMIDOBENZALDEHYDE (EHRLICH'S ALDEHYDE) REACTION OF THE URINE.

By C. A. HERTER.

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Various observers have noticed that the cherry-red coloration of the urine induced by an acid solution of Ehrlich's aldehyde is apt to be particularly strong under some pathological conditions and personal experience has led me to consider these pronounced examples of the reaction especially frequent in affections of the digestive tract. A satisfying explanation of the chemical basis of the reaction is still wanting and the difficulty in supplying it is due in part to the circumstance that this highly reactive aldehyde forms cherry-red compounds in the urine with more than one substance. Thus Bauer has brought forward evidence that the reaction depends on the urobilinogen[1] of the urine and I have shown that the reaction is at least augmented by the administration of skatol. I now have evidence that indolacetic acid is one of the urinary constituents that may react with the aldehyde, although not strongly except in concentrated urines. It is not improbable that there are other ways in which a typical dimethyl-amidobenzaldehyde color reaction may be obtained in the urine. Until all the possible origins of the reaction are known, no satisfactory general statement can be made in respect to any pathological significance which the exaggerated reaction may at times possess. Meanwhile it seems worth while to mention the important influence of a meat diet upon the Ehrlich reaction, for unless this be realized there is a likelihood of falling into the error of ascribing a pathological meaning to a phenomenon which is at times in reality physiological in nature. As patients with

[1] It is probably this fact that has led Fr. Müller to regard the reaction as connected with hepatic disease. I am told that in Müller's clinic the strongest reactions have been noted in diseases of the liver, in which the aldehyde reaction is frequently obtainable in the cold.

disorders of intestinal digestion are often given a diet containing considerable meat, it is easy to see how this fact may complicate the interpretation of a strong aldehyde reaction.

Attention was first attracted to the influence of meat by the fact that a meat-fed dog whose urine gave an intense cherry-red reaction with Ehrlich aldehyde promptly secreted urine which failed to give this reaction when the diet was altered by substituting milk for meat. On returning to a meat diet the urine regained its capacity to react typically with paradimethylamidobenzaldehyde. This observation was repeated with similar results on several dogs. Several trials were then made on men and these trials indicated that an abundance of beef caused an intensification of the aldehyde reaction, whereas a restriction in meat was followed by a distinct decline in the intensity of the reaction, although not necessarily by its abolition.

With a view to finding out what constituent of meat food is concerned with the effect on the urine, three sets of experiments were made. In one of these the milk which served as food was mixed with dog's blood, in order to determine whether the hæmoglobin might be so transformed as to exert an effect on the reaction. No positive effects were obtained from the quantities of blood that were used. In other experiments Liebig's extract of beef was added to the milk. Here there was noted a moderate rise in the intensity of the aldehyde reaction, although the extract itself has no constituent that gives the reaction. These observations were made on dogs. Finally, a healthy man, whose urine habitually gave a strong Ehrlich aldehyde reaction while he was taking a meat diet, was fed ground beef from which nearly all soluble pigments had been washed out by a stream of water. In this case, before beginning the experiment of feeding the colorless meat, the urine was rendered almost irresponsive to Ehrlich's aldehyde by a diet of milk, eggs and bread for a week. On taking one kilo of washed beef in two successive meals, no increase in the reaction was observed, this result contrasting with similar experiments made with unwashed beef in the same quantity. The colorless meat of fish gave results like those obtained with washed beef.

It may be stated that the free use of beef by three healthy men was followed regularly by an intensification of the Ehrlich alde-

hyde reaction of the urine. This intensification is dependent on the presence of the coloring matter of the meat. I hope soon to be able to offer an explanation of this influence of the coloring-matter contained in meat.

It should be mentioned that in the experiments referred to in this note the urines were regularly diluted to a specific gravity of 1010 before testing them. Unless this precaution be taken, there is a liability to error in making comparisons of the intensities of the reactions.

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THE OCCURRENCE OF SKATOL IN THE HUMAN INTESTINE.

BY

C. A. HERTER.

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THE OCCURRENCE OF SKATOL IN THE HUMAN INTESTINE.

By C. A. HERTER.

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The color reaction between paradimethylamidobenzaldehyde (Ehrlich's aldehyde) and skatol[1] gives us an easy method of detecting the presence and of estimating the quantity of this putrefactive product. The employment of the method in routine and experimental work upon the digestive tract has led to the accumulation of numerous observations that have an interest both for the physiologist and the physician. It is my wish to bring together here the chief results of these observations.

It seems to be a general impression, at least among writers of text-books of physiological chemistry, that skatol is a regular product of intestinal putrefaction in the feces. Probably this impression is due to the statement made by Brieger to this effect[2]—a statement which requires some modification. Among healthy children under ten years of age I have found the occurrence of skatol in the feces to be quite exceptional whether they live on a milk diet or on a mixed diet inclusive of meat. Among adults also there are many who show no trace of skatol. It is, however, true that one frequently finds a trace of skatol in the freshly voided feces of healthy individuals.

Where there is an excessive degree of intestinal putrefaction there may be a marked increase in the skatol content of the feces. Whereas in persons who are normal (in the sense that they are

[1] On the Separation of Indol from Skatol and their Quantitative Determination, this *Journal*, ii, p. 267, 1906.

[2] "Das Skatol ist ein constantes Bestandtheil der menschlichen Excremente, fehlt aber in denen des Hundes." Ueber die flüchtige Bestandtheile der menschlichen Excremente. *Journ. f. prakt. Chem.*, xvii, p. 124. Reprinted in Nencki's *Opera Omnia*, i, p. 379.

unconscious of any disorder of digestion) one rarely finds more than 0.5 milligram of skatol in 100 grams of fresh feces, the quantity may reach 8 or 10 milligrams in persons who are the subjects of disturbed intestinal digestion. These quantities I have seldom observed and it may be safely stated that it is rare to find more than 5 milligrams in 100 grams, even where putrefactive decomposition is intense. In this respect the skatol content of the lower intestine differs from the indol content, which is sometimes greater than is represented by the above figures. Moreover it is true that among apparently normal individuals it is more common to find traces of indol in the feces than traces of skatol. To this rule there is observed an occasional exception in which skatol can be detected but not indol.

The detection of skatol in the feces in the absence of indol of course does not prove that there has been no production of indol in the gut, since it may have been formed at some level above the rectum and subsequently absorbed entirely. It is clear that this might be the case where we find skatol but no indol, despite the fact that indican is present in the urine and cannot be ascribed to any but an intestinal origin. The presence of skatol without indol in the lower part of the bowel might be ascribed either to a more ready absorption of indol than of skatol (assuming them to be produced in equal abundance at the same level), or to a relatively late production of skatol.

The following experiment was made with a view to determining whether the absorption of indol from the intestine is more rapid than that of skatol.

Into a loop of dog's ileum which had been previously washed out with salt solution there was introduced 100 cc. of a solution prepared by dissolving 5 mg. of indol and 5 mg. of skatol in 2 cc. of alcohol and diluting to 250 cc. with physiological salt solution. After the introduction of this fluid, the gut was returned into the abdominal cavity. The animal was killed at the end of 35 minutes, when the gut had in a large degree emptied itself. 26 cc. of fluid were found in the gut. After filtration this fluid was distilled and the distillate tested for skatol and indol by means of Ehrlich's aldehyde. By means of the color reaction obtained, it was possible to form a judgment as to the proportions of indol and skatol present in this fluid, a portion of the originally prepared solution being employed as a control in making this comparison. It was found that the tints obtained from the distillate from the gut were so nearly duplicated by

the tints obtained from the original solution that they could not be distinguished. Although there was this close correspondence in the colors obtained by the action of the aldehyde there was a less close correspondence between the colors obtained on shaking out with chloroform, the distillate from the fluid of the gut yielding a reddish rather than a purple tint, a result which may perhaps be regarded as pointing to the presence of slightly less skatol than indol. It was noticeable that the concentration of the indol and of the skatol in the fluid of the loop was less than one-quarter as great (judging by the intensity of the color reaction) as that of the original solution.

It is evident from the foregoing experiment that it gave no indication that indol is absorbed more readily than skatol—a result which harmonizes well with what is known of the close resemblance between indol and skatol in respect to solubility and chemical constitution. It thus appears in a high degree improbable that differences in the rate of absorption of indol and skatol from the intestine can account for the preponderance of skatol over indol that is sometimes noted in the contents of the human colon.

There is some evidence that skatol is in general a later product of putrefaction than indol. Nencki recommends long standing putrefactive mixtures containing muscle fiber and pancreatic gland, in order to obtain skatol in fair quantities.[1] I have many times noticed in the course of putrefactive experiments *in vitro* that skatol appeared several days later than indol. It is not certain that this fact helps to explain the finding of skatol at lower levels of the intestinal contents than indol, because the conditions of decomposition within the intestine are so different from those that are experimentally induced. Experiments made on human subjects with the aid of cathartics indicate that proteid food may yield skatol within 24 hours, whereas, on artificial culture media, I have never observed it before the lapse of several days. Still it is likely that although both indol and skatol are sometimes more rapidly formed in the intestinal tract than in experiments *in vitro*, the relatively later formation of skatol is also a feature here. This seems the most reasonable explanation of the occurrence of skatol in the feces without indol, in those cases where indol has certainly been formed and absorbed.

[1] Vortheilhafte Darstellung des Skatols, *Centralbl. f. d. med. Wiss.*, No. 47, p. 849, 1878; *Opera omnia*, p. 433.

As yet I have not had a sufficiently long experience to make a generalization with respect to the clinical conditions under which the intestinal contents persistently show the presence of skatol in excessive quantities. I have not found skatol abundant and persistent in the feces except in the case of persons who are ill or have recently been ill of some intestinal disorder. For this reason I have come to attach to its presence more significance than to the presence of indol, which is not infrequently found in the intestinal contents of persons in apparently good health, who are unconscious of any digestive disturbance. As to the kind of proteid food most apt to favor the production of skatol no definite statement can be made beyond the fact that a milk diet does not necessarily cause skatol production to cease, although it seems to render it less active than where the diet contains an equal quantity of nitrogen in the form of meat proteid.

Where skatol formation is active in the intestine there are usually other indications of excessive putrefaction, especially an increase in the ethereal sulphates of the urine and an excessive formation of hydrobilirubin within the gut. Indol production is usually excessive but I have observed instances in which this was not the case, instances in which the excessive putrefaction was skatolic rather than indolic in type. This is not usually a transitory phenomenon but is likely to be a long persistent feature.

The excessive formation of skatol I have found especially in what I have described as instances of chronic excessive saccharo-butyric intestinal putrefaction. Mental or emotional depression has in some of these cases been the most persistent clinical feature; in others there has been a moderate or considerable degree of simple anæmia. In still others there have been present the blood changes characteristic of pernicious anæmia. I have come to believe that the chronic digestive disturbances of pernicious anæmia are almost regularly associated with an excess of skatol in the feces. In a case of appendicitis which came under my observation the feces contained skatol for several weeks after an operation for removal of the appendix; with return to health this substance gradually disappeared. In a patient with multiple neuritis associated with persistent constipation and great production of intestinal gases, skatol was regularly obtained in abundance from the feces during the period of paralysis. With gradual convales-

cence there was a complete disappearance of the skatol of the feces. Several other examples could be mentioned which illustrate the disappearance of skatol from the feces, concomitantly with a betterment in clinical conditions. Apparently it would be worth while to make careful systematic observations on the quantities of skatol obtainable from intestinal material derived from a variety of patients suffering from intestinal disease, with a view to learning in how far the presence of this substance is a guide to the intensity and course of bacterial processes in the intestinal tract.

In practice I have found it convenient to make use of the following mode of procedure in examining human feces for skatol. Twenty grams of fresh material are ground in a mortar with a convenient quantity of water. The suspension is then diluted up to 300 cc. It is now acidified with phosphoric acid and distilled, the distillation being continued until it no longer gives any color reaction with paradimethylamidobenzaldehyde. A portion of the distillate is now treated with a paradimethylamidobenzaldehyde solution made up by dissolving 15 grams of the aldehyde in 30 cc. of concentrated sulphuric acid and diluting this to 100 cc. The aldehyde solution thus prepared is added to the distillate until the maximum color reaction appears. This may be somewhat heightened by the addition of a small amount of concentrated hydrochloric acid. If indol is present in the distillate it should be removed by means of β-naphthaquinone-sodium-monosulphonate.[1] If phenol is present it must be got rid of by redistillation, it being held back by strong alkali. For clinical purposes the quantity of skatol present may be closely enough approximated by comparing the color obtained with Ehrlich's aldehyde with various dilutions of a watery solution of skatol of known strength. It is best to make the comparison after the contents of the test-tube have cooled, as this causes a deepening of the color toward blue.

I have made experiments with many kinds of bacteria (using pure cultures of bacteria and also using mixtures of bacteria) in the hope of learning something of the conditions under which skatol is formed rather than indol. Only a fair measure of success has been gained in this attempt. The main obstacle to success is the difficulty in obtaining a culture medium really comparable to that which the skatol-making bacteria find within the human intestine. If we inoculate ordinary culture media (peptone bouillon, plain agar, blood agar, milk) with mixed fecal

[1] A Method for the Quantitative Determination of Indol, this *Journal*, i, p. 257, 1906.

acteria from a specimen containing skatol we are almost certainl isappointed in the hope that skatol will result in the course o he subsequent incubation. The putrefactive decompositio ields indol, not skatol. Only in rare instances is a trace of ska ..ol found. From fluid media containing ground brain substanc or fibrin one may obtain skatol, but the yields are seldom con siderable and the putrefaction is usually indolic rather than skatolic Sometimes after long putrefaction there is a considerable yield c skatol.

Difficulties have also been encountered in securing pure cul tures of microörgani e of regularly giving rise to skatc when grown upon o ure media. Marked irregularitie in skatol formatio ..e ne instances been observed wher it has not been possibl rately determine what condition have been responsible se irregularities. Nevertheless few organisms out of a l mber that were tried were found t produce skatol with con able regularity when grown o peptone bouillon to whic d has been added. These organ isms were a strain of ma : œdema obtained from Prof. Theo bald Smith, two strains o *putrificus* and an unidentified putre factive anaërobic organism sent me by Dr. Smith. It is note worthy that the best success in obtaining skatol has come from th use of organisms that grow under anaërobic conditions. Som strains of *B. proteus* of Hauser are probably also to be regarde as skatol-producers. There are doubtless many other anaërobi bacteria, besides those mentioned, which are capable of formin skatol. I have not been able to satisfy myself that *B. aërogene capsulatus* ever produces skatol, though some strains make indol As to tetanus, I am also in doubt. I have never been able t obtain more than mere traces of skatol from any strain of colo bacillus in my possession, whether the organism was grown aëro bically or anaërobically. Long cultivation was required to giv even these insignificant traces.

It is certain that the conditions which lead to the formation o indol are fundamentally different from those leading to the pro duction of skatol. The conversion of skatol into indol is one tha might be thought of as possible for microörganisms to effect I have made experiments with a view to determining whethe colon bacilli which were energetic indol-formers could form indo

from skatol. The organisms were grown on a medium consisting of gelatin and salts to which a small quantity of skatol had been added. The organisms grew abundantly on this medium but even after many weeks' growth not a trace of indol could be detected. This result is in harmony with the observations made by Ellinger[1] on indol and skatol formation in the intestinal tract of the rabbit. He states that at most a mere trace of indol may possibly be formed from skatol. I question whether even this is likely. Indol has been produced from skatol by potash fusion but so severe and destructive a method as this cannot be compared with any powers which it is likely that bacteria can exert.

That both indol and skatol are derived from tryptophan cannot be doubted. One may easily satisfy oneself of the ability of trytophan to yield skatol and indol by experimenting with media made up by the addition of tryptophan to gelatin, pure cultures of different bacteria being employed. There is no reason to suppose that any other constituent of the proteid molecule than tryptophan is able to yield indol and skatol. We have thus to look to the chemical constitution of tryptophan for a clue to the solution of the problem why skatol is sometimes formed and at other times indol. I cannot pretend to offer an adequate hypothesis upon this question, but would like to call attention to certain facts which point to the reasonableness of the idea that the formation of skatol in one case and the formation of indol in another, may really be conditioned by the nature of the intermediate products that arise before tryptophan can yield either one of these substances. It may now regarded as settled that tryptophan is indol-amidopropionic acid and not skatol-amidoacetic acid, as was thought more likely by Hopkins and Cole. Moreover it is probable that tryptophan is an α-amido acid. It is certain that under the action of microörganisms tryptophan is capable of yielding indol-acetic acid, as was shown by Hopkins and Cole[2] to be true of a tryptophan medium inoculated with a pure culture

[1] Ueber die Constitution der Indol Gruppe im Eiweiss (Synthese der sogen. Skatolcarbònsaüre) und die Quelle der Kynurensäure, *Ber. d. deutsch. chem. Gesellsch.*, no. 7, p. 1802, May 4, 1906.

[2] Called by them skatol carbonic acid. The Constitution of Tryptophane and the Action of Bacteria upon it. *Journ. of Physiol.*, xxix, p. 438, 1903.

of *B. coli communis*. This substance is probably not readily attacked by microörganisms. Some bacteria appear unable to attack indol-acetic acid. Thus *B. coli communis*, although able to form this substance from tryptophan either makes no skatol from it or only minute quantities. It is however probable that some bacteria (especially putrefactive anaërobes) are able to act upon indol-acetic acid in such a way as to cause it to lose carbon dioxide and such a change would explain the production of skatol. Assuming indol-acetic acid to be relatively unattackable by microörganisms, one would have an explanation of the usually small and slow yield of skatol in putrefaction.

Decomposition of tryptophan may, however, take another direction. Through oxidation, with the removal of the amido group, indol-propionic acid is formed. Assuming that this substance is relatively easily attacked by microörganisms, it is easy to see how indol and carbon dioxide might result from such bacterial attack, indol-carbonic acid being formed as an intermediate product. There is apparently no reason to suppose that indol-acetic acid is readily converted into indol-carbonic acid, since this calls for a process of energetic oxidation only likely to occur through the action of relatively powerful oxidizing agents. It would appear, then, that the formation of skatol may hinge on the antecedent production of indol-acetic acid, whereas the formation of indol may depend on the production of indol-propionic acid. To what extent this suggestion may be borne out by experimental facts can only be determined by further observations. Dr. Dakin has been so kind as to offer to prepare for me these important intermediary substances, the possession of which should serve to definitely determine their bacterial relation to indol and skatol.

The main conclusions which I desire to emphasize are the following:

1. Skatol is by no means always present in the contents of the lower gut in man. In healthy children it is only seldom detectable and then only in traces. In healthy adults it is frequently absent and when present occurs only in traces.

2. In some cases of excessive intestinal putrefaction skatol formation is considerably increased, often together with increased indol formation but sometimes without this.

3. There are instances in which the feces contain skatol but

no indol, despite the fact that the presence of indican in the urine points to indol formation in the intestine. As there is no evidence that indol is absorbed more rapidly than skatol in such cases, the presence of skatol without indol is probably due to the later production of the skatol.

4. Increased skatol production is observed in many persons suffering from excessive saccharo-butyric putrefaction due mainly to putrefactive anaërobic bacteria.

5. There are strains of the bacillus of malignant œdema and of *Bacillus putrificus* which form skatol. The *Bacillus coli communis* makes indol but usually no skatol or only mere traces.

6. The conditions giving rise to the formation of skatol are fundamentally different from those that govern the formation of indol. The formation of indol-acetic acid is perhaps a necessary step in the production of skatol, most bacteria attacking it with difficulty, if at all.

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ON THE THERAPEUTIC ACTION OF FERMENTED MILK

By C. A. HERTER, M.D.

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ON THE THERAPEUTIC ACTION OF FERMENTED MILK

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DURING the past year there has been in the United States a large increase in the use of fermented milk in the treatment of disorders of digestion and nutrition. A clearly discernible influence in bringing about this increase lies in the publications made by Professor Metchnikoff and his colleagues in reference to the fermented milk known as lacto-bacilline. The statements made by these scientific workers have been repeatedly exaggerated by persons having a commercial interest in the sale of certain kinds of fermented milks. It is apparently true that many physicians have been influenced by these statements in the direction of recommending among their patients a much wider use of fermented milk, and especially of lacto-bacilline, than was previously the case. Moreover many persons have decided without the advice of a physician to make a trial of some form of fermented milk or of some form of lactic acid ferment capable of acting upon milk sugar. It appears that this dietetic practise is still on the increase and likely to modify the habits of a not unimportant part of the community in respect to diet. In view of this fact it seems to me desirable to consider from a critical standpoint the therapeutic effects supposed to be derivable from the use of fermented milks, and more especially from milk that has been fermented through the use of the *B. bulgaricus* recommended by Professor Metchnikoff and now widely employed in the production of lacto-bacilline. I believe that at the present time there exists a considerable confusion of mind as to what may or may not reasonably be expected in the way of therapeutic results from the use of milk which has undergone lactic acid fermentation. It is the object of this paper to consider briefly the elements which should enter into the formation of a judgment as to the therapeutic efficacy of lacto-bacilline and allied milk products.

In order to be able to form an unimpeachable judgment on the therapeutic action of a fermented milk, it is necessary that experiments of a very painstaking sort should be carried on in a number of individuals for considerable periods of time. Experiments of a kind calculated to furnish a firm scientific foundation for a rational use of fermented milks have not yet been made. Such experiments in order

to be decisive would have to be conducted not merely on people in good health, but also on a suitable variety of digestive and nutritional disorders in which the bacterial conditions in the intestine, the state of metabolism and the general conditions of life are taken into account with the greatest care and judgment. Although I have for many years been interested in watching the influence of fermented milk on the human organism in various states of digestive derangement, and have accumulated many observations bearing on the question, my experience falls far short of what is necessary to establish final conclusions. In this communication, therefore, I do not offer any solution of the therapeutic problems pertaining to the use of fermented milks, but seek only to discuss critically, in the light of such information as now exists, some of the claims that have been made for the employment of these kinds of milk. I do this with the thought that a discussion of the various elements which should enter into the formation of a judgment regarding the therapeutic value of milk subjected to lactic acid fermentation may prove helpful to those who have not given the subject much personal study and are therefore unable to analyze the problem in a way that is likely to serve as a practical guide.

There are five important kinds of effects referable to the action of fermented milks which must be considered in any judgment of the therapeutic effects of a milk which has undergone lactic acid fermentation. These are, first, the effects on the absorption of fats and proteins; secondly, the effects due to reduction of carbohydrates; thirdly, effects due to the presence of lactic acid; fourth, effects due to the bacteria used in lactic fermentation; fifth, effects due to a lowering of putrefactive decomposition. These latter effects, which are of the first importance in connection with any study of the action of fermented milk, are of course not entirely distinct from the others just mentioned, but stand related to each of these other factors. Owing to their prominence, however, it is desirable that they should be separately considered.

At present the influence of lactic acid fermentation upon absorption of the milk constituents is but little understood. The question relates especially to the absorption of fats and of proteins, for the carbohydrates of the milk are in large degree removed by the fermentative process, lactic acid, carbon dioxide and alcohol being the chief constituents resulting from the breakdown of the milk sugar. It is important that we should obtain exact data with regard to the absorption both of the fats and of the proteins, but, so far as I am aware, these do not at present exist. If it could be shown that the absorption of milk fat and of milk proteins is increased in health through the influence of lactic acid fermentation of any kind, this would be a distinct argument in favor of the use of such milk as an article of diet,

since it would make for economy in the administration of the machinery of the body. Equally important and desirable are reliable observations on the effect of fermented milks on the absorption of milk fats and milk proteins in various types of intestinal infection with their accompanying acute and chronic catarrhal inflammation of the mucous membranes of the digestive tract. The therapeutic claims put forward by enthusiastic advocates of the use of fermented milk have in general taken a different direction and have concerned themselves much more with the question of the reduction in intestinal putrefaction than with increase in absorption. But it must not be overlooked that an improved absorption of proteins is one of the most important conditions in general for reducing intestinal putrefaction, because whatever favors prompt and complete absorption must correspondingly limit the opportunity for decomposition. In a lesser degree this statement holds true also of the fats. I have been able to show experimentally that in normal persons the butter-fat may be much increased above the usual intake—say from fifty grams to one hundred and fifty grams daily—without materially increasing putrefactive decomposition. On the other hand, such an increase in butter-fat in persons already suffering from increased putrefactive decomposition shows a pronounced tendency to still further increase the putrefaction. I attribute this tendency to the mechanical obstacle to prompt absorption of proteins arising from the presence of fat in abundance. The failure in prompt absorption of proteins from an intestine infected with putrefactive microorganisms means intense putrefaction, whereas a similar failure in a healthy intestine is far less significant owing to the relative infrequency of putrefactive bacteria.

In considering the therapeutic influence of fermented milk, it is necessary to take into account the fact that in such milk the carbohydrate material has been in a large degree replaced by the products of fermentation. Where milk is used in only small amounts in the dietary, and these small amounts are replaced by a fermented milk, the difference in quantity in respect to the intake of carbohydrates may be so small as to be negligible. Where, however, the dietary consists largely of milk and this large amount of milk is replaced by an amount of fermented milk equivalent in protein and in fat, the difference in respect to the carbohydrate material may assume considerable importance. In the case of the unfermented whole milk, there is enough milk sugar to markedly encourage fermentative decomposition in the intestine with the production of considerable gas. The gas-forming organisms especially likely to attack the milk sugar are *B. lactis ærogenes*, *B. coli* and *B. ærogenes capsulatus* (*B. welchii*, or *B. perfringens*). In cases where there is marked flatulence from the use of whole milk, the use of any fermented milk in which the milk sugar

has been largely destroyed by fermentative bacteria introduces conditions unfavorable for intestinal fermentation. I consider that the diminution in fermentable material thus arising from the decrease in the carbohydrates of the milk is an important factor not merely in reducing intestinal fermentation, but also in reducing intestinal putrefaction, for it is true that in some intestinal infections in which we are justified in assuming that the colon bacillus or *B. ærogenes capsulatus* or both these organisms have extended in an upward direction toward the stomach, the abundant presence of fermentable carbohydrate pabulum leads to a great increase in these microorganisms. After the absorption of the acid produced in the course of this fermentation there may be established a neutral or even an alkaline reaction in the lower part of the small intestine and in the colon. In the absence of acid and indeed in the presence of a moderate amount of acid, the colon bacilli and *B. ærogenes capsulatus* are capable of making an increased attack upon the protein material. This increases intestinal putrefaction. On the other hand, the irritation arising from organic acids formed in the small intestine and stomach often leads to a fermentative diarrhœa.

Turning now to the effects attributable to the presence of lactic acid in the soured milk, it is at once apparent that we have to distinguish clearly between the action of such preformed lactic acid as may be introduced with the milk and such acid as may be formed in the course of further lactic acid fermentation after the soured milk has been ingested. The essential difference lies in the fact that such lactic acid as is preformed in fermented milk is liable to be absorbed from the upper part of the small intestine, whereas if lactic acid fermentation goes on within the digestive tract, the acid may be formed at any level of the intestine. In the former case the action of the acid is to be regarded as largely limited to the portion of the intestine in which putrefactive decompositions seldom occur; in the latter case there may be production of acid within the territory in which putrefactive decompositions are apt to take place. We should therefore expect greater anti-putrefactive efficacy from the use of soured milk containing living lactic acid producers than from the same milk after sterilization. Whether such a difference as this is actually discernible in practise I am unable to say, as I am not aware of the existence of satisfactory experiments made to test this point.

As to the efficacy of lactic acid as an anti-putrefactive agent it is necessary to speak with caution. It has been the practise of many physicians to employ lactic acid in the treatment of disorders of digestion, especially those of infancy. But I am unaware that we have adequate data for the establishment of the therapeutic anti-putrefactive value of lactic acid. Where the stomach secretes no free hydrochloric

acid it is reasonable to suppose that the use of lactic acid in weak concentration exerts some anti-fermentative action, especially against such microorganisms as do not readily grow in acid medium. But there are many kinds of microorganisms in the digestive tract which are resistant to the action of lactic acid in the low concentration which can be tolerated by a somewhat irritable mucous membrane. Most yeasts and some important intestinal bacteria, such as *B. lactis ærogenes, B. bifidus, B. infantilis* and various organisms classed at acidophiles, have this property. It is a fact little known that some of the coccal organisms of the intestine resist the action of acid in a remarkable measure. It is therefore quite clear that anything approaching a significant modification of the activities of organisms of the types just mentioned is not to be looked for through the use of lactic acid. Moreover, I have shown that a considerable grade of acidity in the intestinal tract is consistent with very active fermentative growth of *B. ærogenes capsulatus*. This organism forms butyric acid during the fermentation of carbohydrates, together with only small quantities of lactic acid, and there is no reason to suppose that its development in the intestine is materially inhibited by any concentration of lactic acid which is likely to be obtainable in the lower part of the small intestine or in the colon, either as the result of administering lactic acid or in consequence of the use of soured milk.

That a considerable or high degree of putrefactive decomposition in the intestine is not controllable in man by the administration of moderate doses of lactic acid has become plain to me as the result of clinical observation. And that even very large doses of lactic acid are unable to restrict intestinal putrefaction is rendered highly probable from experiments made in my laboratory by Dr. Helen Baldwin. In dogs taking a meat diet and excreting urine characterized by abundant indican and high ethereal sulphates there was no falling off in putrefaction as a result of administering doses of lactic acid as large as five grams daily. It seems to me doubtful if under these circumstances enough lactic acid could reach the large intestine to exert even a moderate anti-putrefactive action. The experiments just mentioned represent an extreme case, since they were made on animals living exclusively on meat. The results obtained can not, therefore, be regarded as strictly applicable to man. Nevertheless these experiments are instructive as indicating the inefficacy of large doses of lactic acid in controlling intestinal putrefaction where the conditions for such putrefaction are favorable and where the acid is given under conditions rendering likely its absorption in the upper part of the digestive tract.

That the presence of lactic acid in soured milk does not necessarily exert a significant anti-putrefactive action in the large intestines is clearly shown by the observation which I have several times made that

persons suffering from chronic intestinal putrefaction have shown no diminution in the putrefactive products excreted in the urine where the patients have added a soured milk to their usual diet. It is, of course, clear that in cases of this sort the failure of the putrefactive process to decline may be attributable to the introduction of more than the habitual amount of protein material. The observation is, however, of interest in that it emphasizes the fact that the ingestion of lactic acid, even if probably associated with lactic acid fermentation within the intestine, may not suffice to exert any beneficial influence in reducing putrefaction.

I do not wish to be understood as maintaining that the presence of lactic acid in soured milk is of no value in checking intestinal putrefaction. I wish merely to point out that the administration of lactic acid *per se* can not be regarded as a significant anti-putrefactive procedure. It seems to me probable, on the other hand, that the presence of lactic acid in the large intestine would at least in a degree tend to restrict putrefactive decomposition. But I must own that positive evidence on this point seems to be at the present time entirely wanting. In my judgment only very carefully planned studies would suffice to enable us to form a final opinion on the value of lactic acid as an anti putrefactive agent. We are not justified in developing an enthusiastic attitude toward lactic acid as an agent in the inhibition of intestinal putrefaction on the basis of our present knowledge.

Let us now consider the effects derivable from the bacteria used in lactic fermentation. As an example of a strong lactic acid producer we may take *B. bulgaricus,* used in the production of lacto-bacilline. This organism is a powerful lactic acid ferment, forming large amounts of lactic acid from milk sugar while forming very little alcohol. The organism grows well in milk and on some media containing an abundance of soluble carbohydrates, as, for instance, in malt extracts. We may take the behavior of *B. bulgaricus* in the digestive tract as being typical of efficient lactic acid bacilli in general. There are two questions which we must put to ourselves regarding the therapeutic effects of such bacteria. First, to what extent do the lactic acid bacilli replace obligate normal types of bacteria or the undesirable saprophytic forms present in disease? Secondly, to what extent is it desirable that there should be a replacement of the intestinal flora by lactic acid bacilli?

It is one of the fundamental assumptions of the sour milk treatment of intestinal diseases that the lactic acid producing microorganisms establish themselves throughout the digestive tract and through their more or less aggressive growth directly or indirectly inhibit the development of putrefactive or other undesirable forms of bacteria. In some of the statements put before the public in regard to the action

of the lactic acid bacilli it is claimed that they drive out other forms of bacteria from the large intestine, the chief seat of intestinal putrefaction. It is desirable that we should soberly consider the known facts relating to this question. I think it safe to say that the ability of lactic acid forms to replace or dominate other types of bacteria in the large intestine is much exaggerated. I have devoted some study to this question, especially in the case of the *B. bulgaricus* employed in the production of lacto-bacilline. This organism, owing to its large size, morphology and cultural peculiarities is easily recognized and is cultivable, from the intestinal contents. When given to human beings in the large numbers present in lacto-bacilline it can after a few days' administration be cultivated without difficulty from the movements. Even when large quantities of the fermented milk have been taken I have not found that it becomes the dominant organism, although it may be present in moderate numbers. On stopping the administration of the lacto-bacilline, the *B. bulgaricus* generally disappears in the course of a few days, showing that it has not permanently established itself within the intestinal tract. There may be exceptions to this statement, but I have not yet met with any. These clinical results are quite in accord with those obtained by Dr. Kendall and myself in experiments upon a monkey fed for two weeks on lacto-bacilline exclusively. At the end of this period, when the movements were showing the regular presence of *B. bulgaricus* in relatively moderate numbers, the animal was killed and the digestive tract examined with care at all its levels. The lactic acid organisms were found in greatest abundance in the small intestine. In the lowest portion of the small intestine a notable falling off was observed and other types of bacteria were prominent. In the large intestine the numbers were only moderate as compared with other varieties of bacteria, thus clearly showing that in this instance, at least, the *B. bulgaricus* was very far from dominating other associated types of bacteria. I consider this fact noteworthy, as the experiment was carried out under conditions highly favorable to the establishment of the lactic acid bacilli in the digestive tract. The large number of microorganisms given and the relatively short extent of the digestive tract in the monkey should, it would seem, provide conditions for the adaptation of the organisms throughout the alimentary canal.

It is probable that the experience just recounted with regard to lactic acid bacilli is not at all exceptional, or in other words that foreign bacteria in general find it difficult to gain a permanent footing in the digestive tract. The literature of experimental bacteriology shows this to be the case. Personal experiments made with a highly fermentative putrefactive organism—*B. ærogenes capsulatus* (*B. welchii* or *B. perfringens* of the French writers)—in feeding experi-

ments on monkeys showed that in health these animals have the power of very quickly ridding themselves of this variety of bacteria. Experiments now under way with a microorganism described by myself and Dr. Kendall as *B. infantilis* and found very abundantly in some of the digestive diseases of children, show the same thing to hold true.

The fact that *B. bulgaricus* does not readily gain a dominant position in the digestive tract in man or in the monkey has an obvious bearing on the results to be expected from its therapeutic use. If it be indeed true that *B. bulgaricus* is capable by its presence in the intestinal tract of inhibiting undesirable types of bacteria and especially the microorganisms concerned with intestinal putrefaction, then it must be equally true that the difficulty in [illegible]ng a dominant and permanent foothold in the intestinal tract is a [illegible] with which we must reckon in any estimate of the results likely to be [illegible]tained through the administration of these organisms. The moderate representation of *B. bulgaricus* in the large intestine after the free administration of lacto-bacilline is surely something very different from what has been already frequently pictured by the enthusiastic upholders of the use of this form of fermented milk in the treatment of diseases of the digestive tract.

I think it has been assumed with far too little reason that the dominant presence of foreign microorganisms of the lactic acid group is necessarily a desirable thing. If it could be shown that lactic acid bacilli, such as *B. bulgaricus* or certain varieties of *B. acidi lactici*, have the faculty of replacing undesirable forms of microorganisms such as the bacilli of typhoid or of paratyphoid fever or putrefactive microorganisms, such as *B. proteus vulgarus* or *B. ærogenes capsulatus*, this would undoubtedly be cause for congratulation, especially if it could be shown at the same time that the normal flora of the digestive tract remained unchanged. I do not deny the possibility that this selective kind of anti-bacterial action may some day be proved to exist. I desire merely to point out that at present I know of no facts to justify us in believing that such antagonistic action as the lactic acid bacteria may possess is directed solely against the disease-inciting invaders of the digestive tract. If it should prove true that the antagonism exerted by the lactic acid bacilli against injurious invaders is also exerted against the obligate bacterial inhabitants of the alimentary canal, such as *B. coli communis* and *B. lactis ærogenes*, I am by no means convinced that this could be regarded as a point in favor of the prolonged therapeutic use of lactic acid bacilli. If, as appears to be true, these obligate inhabitants of the digestive tract are especially adapted to the normal conditions of secretion and digestion in the human intestine and tend to be suppressed in some serious conditions of the digestive tract (while their reappearance and reestablishment in [illegible]umbers

is one of the first, most definite and most reassuring signs of improvement in the clinical condition of certain kinds of patients) to make use of any mode of lactic acid bacillus therapy which will inhibit the normal development of *B. lactis ærogenes* or *B. coli communis* in the digestive tract would, in my judgment, be a profound error in principle. I do not wish to intimate that I consider *B. bulgaricus* or any of the common lactic acid bacilli to be capable of seriously checking the growth of *B. lactis ærogenes* and *B. coli communis* in the digestive tract, but wish to state that in so far as such modification is possible it appears to me not without undesirable features. To the validity of this statement there is one possible exception that occurs to me. In cases where there is a colon bacillus infection of the intestine, that is to say, an inflammatory state associated with a great over-growth of *B. coli*, the antagonistic influence of lactic acid bacilli might be useful. But I am not sure that this is more than a merely apparent exception to the general rule which I have above expressed as valid, for it is not clear that it has been proved that the colon bacilli apparently answerable for digestive infections are in reality the normal colon bacilli. It appears to me more likely that they are commonly variants of such bacilli whose fermentative characters have not yet been determined fully and precisely.

I would also mention here the fact that there are diseases of the intestinal tract associated with the presence of bacteria capable of forming lactic acid. Obviously, then, this property of a microorganism does not necessarily screen the digestive tract from injury.

One of the most important and most loudly heralded effects of the administration of soured milk is that on intestinal putrefaction. Under conditions of health the putrefactive decompositions in the intestinal tract seldom attain a considerable degree of intensity—a surprising fact when we consider the immense numbers of bacteria which inhabit the large intestine. In many pathological states the conditions of putrefaction in the intestine are very much altered in the direction of great intensification. This is shown both by the dominance of putrefactive microorganisms in the large intestine and by the appearance of products of putrefaction in the urine. It is unnecessary here to discuss the nature of these products. It should, however, be pointed out that the intensity of putrefaction as judged by the quantity of putrefactive products in the urine is notably influenced by the quantity of protein material ingested. We may say that in general a considerable increase in the protein intake is followed by a corresponding increase in putrefaction and that a marked diminution in protein intake is followed by a distinct falling off in putrefaction. This statement holds true in general in conditions of health and it is even more strikingly exemplified in cases of chronic intestinal infection associated

with habitual excess in putrefaction. In view of this fact it is clear that in experiments designed to determine the influence of fermented milks upon the intensity of putrefaction it is essential to take accurate cognizance of the quantity of protein ingested. It is easy to understand that if a patient has been in the habit of eating for his midday meal an abundance of protein food and decides under advice to take a fermented milk for his lunch in place of the more elaborate meal, the mere reduction in protein will suffice to reduce putrefaction. So it is clear that a decrease in putrefaction can be effected through a variety of dietaries which have in common the fact that they contain a smaller amount of protein material than the patient has been in the habit of eating. Whole milk and various fermented milks are thus capable of influencing putrefaction in such a way that we may readily fall into the error of exaggerating their influence upon putrefactive decomposition in the intestine. Hence it is evident that the only fair test of the value of a fermented milk in respect to its influence on putrefaction is to compare it with the effects of other articles of diet containing exactly the same amount of protein material. Such careful comparisons have not, I believe, been made up to the present time. In the future they will doubtless be made and will enable us to form quite definite judgments as to the relative effectiveness of different kinds of fermented milks upon intestinal putrefaction. At present I should hesitate to say that one kind of fermented milk is more effective than another in bringing about a reduction in intestinal putrefaction.

We may regard it as well established that a diet in which milk takes the place of other kinds of food is very apt to be followed by a reduction in the intensity of putrefactive decomposition in the intestine. There are, however, clinical indications that the use of fermented milks does possess real advantages over the use of whole milk at least in some disorders of digestion. Although the exact character of these advantages is not yet firmly established, they seem to be none the less real. From what has already been said in this paper on the criteria of judgment of the action of fermented milks, it is evident that the clinical advantages which have been observed may be attributable to several different peculiarities possessed by fermented milks in general. One of these is the favorable mechanical influence on the minute subdivision of the casein, which prevents the undesirable effects associated with the presence of large clots of casein which are not easily disposed of in persons with weak digestion. The exact consequences of this advantageous mechanical state of the milk food can not now be appraised. A second point which has already been mentioned is the formation of lactic acid. Here again the precise extent of the favorable influence can not be measured; but on the other hand it can be denied that in at least some disorders of digestion the presence

of lactic acid in the intestinal tract may exert a degree of anti-putrefactive action. It should, however, be remembered that there are persons with chronic inflammatory states of the digestive tract who tolerate very badly acids of all sorts. These persons are unable to take considerable quantities of fermented milk if the milk contains a high percentage of lactic acid, the attempt to utilize such food being followed by various unpleasant sensations and diarrhœa. The possible anti-putrefactive influence of the presence of living lactic acid bacilli in various parts of the digestive tract has already been discussed at sufficient length and it has been pointed out that this factor again is one whose value can not at present be accurately estimated.

It must be plain from what has been said that the therapeutic use of fermented milks rests at the present time rather more securely on the clinical observations that have been made with it than on an adequate scientific study of the influence exerted upon digestion and nutrition and especially on the processes of putrefaction. To obtain the necessary scientific data will require elaborate and very laborious experiments covering long periods of time. With the aid of such experiments I have no doubt that the usefulness of soured milks in health and in disease will be definitely and discriminatingly established. The limitations of utility will become equally plain, and I predict that they will prove to be many. The importance of this subject for the welfare of people at large not only in respect to immediate physical comfort and efficiency but as regards the prolongation of life, would, in my opinion, amply justify a very considerable expenditure of money to acquire this knowledge.

It can not be regarded as surprising that the enthusiasm which has been aroused partly through the public exploitation of various kinds of fermented milk in the treatment of disease and partly by the undoubted successes of the treatment should have led to various abuses. One of the most important things to understand in reference to the use of fermented milk is that it should be employed in most instances as a substitute for other forms of food rather than as an addition to the usual dietary. Especially is it necessary to bear this in mind in the case of chronic disorders associated with an increase in putrefaction. The addition of a considerable amount of fermented milk to the habitual dietary has often been practised with disastrous results, and I do not doubt that this practise is still widely extended. Such bad results might be predicted, for since all fermented milks contain a large proportion of protein material capable of undergoing putrefaction and since this putrefaction is not checked, in any specific way, through the agency of the fermented milk itself, a great increase of putrefactive decomposition may follow the injudicious excessive use

of such food. I have seen several instances of this error, which is not confined to laymen, but is sometimes committed by physicians also.

Another feature of fermented milk which needs to be closely scrutinized is the character of the microorganisms employed as ferments of the milk. In a few instances I have known to be used as ferments what I believe to be very undesirable types of bacteria. I think it may be said that most of the fermented milks on the market in this country at the present time contain chiefly fermentative organisms which are harmless when not excessively administered. In some cases the lactic-acid producing bacteria have become contaminated by possibly undesirable yeasts. It is only natural that accidents of this sort should occur in what is comparatively a new industry, and it is likely that with increasing experience the manufacturers of the various fermented milks will be compelled to exercise every reasonable caution in regard to the purity and quality of the ferments employed in their products.

The use of tablets of other preparations of lactic acid bacilli is now becoming widespread. The tablets are taken with some carbohydrate material which will permit the growth of the bacteria and the formation of lactic acid. I have seen good results from this method of using lactic acid bacilli, in the relief of symptoms referable to excessive intestinal putrefaction. But I do not think the data exist at present for an intelligent comparison of this use of lactic acid bacilli with their use in fermented milks. I hope before long to be able to discuss this question on the basis of experimental observations.

NOTES ON THE ACTION OF SODIUM BENZOATE ON THE MULTIPLICATION AND GAS PRODUCTION OF VARIOUS BACTERIA

BY
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NOTES ON THE ACTION OF SODIUM BENZOATE ON THE MULTIPLICATION AND GAS PRODUCTION OF VARIOUS BACTERIA.

BY C. A. HERTER.

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In the course of the summer of 1908, while carrying on a research on the action of sodium benzoate on the human body, which has been published in Bulletin No. 88 of the United States Department of Agriculture, observations were also made upon the influence of sodium benzoate upon the multiplication and gas production of various kinds of bacteria, and some of these observations appear to be of sufficient interest to make it worth while to place them on record, especially in view of the interest that has been roused recently through the discussion of the influence of sodium benzoate upon the human organism. The observations upon the effect of sodium benzoate on the multiplication and gas production of bacteria, which form the subject matter of these notes, do not constitute a systematic examination of the subject in question, but they are sufficiently numerous and varied to establish some features of interest which I think have not been heretofore noted.

I. One portion of the work related to the examination of various canned and bottled foodstuffs and condiments. At my request, made through the secretary of the National Food Manufacturers' Association, thirty-seven different varieties of canned and bottled foods were sent to me for examination. This list does not include certain samples of codfish which had been spoiled through the action of bacteria, which were sent to me from Gloucester, Mass. The food-stuffs and condiments sent me included samples of chili sauce, catsup, sweet pickles; table syrup; peach, blueberry, strawberry, damson, blackberry, apricot, pineapple and white cherry preserves; strawberry, blackberry, apricot, pineapple and white cherry jam; currant

jelly; lemon and prune pie-filling; apple cider; and various soda fountain syrups.

The procedure followed in the examination of these foodstuffs was to introduce about 0.5 gram of the solid or semi-solid material into dextrose, lactose and saccharose bouillon which had been sterilized in fermentation tubes. In the case of fluid preparations 0.5 cc. was added to similar fermentation tube media. All the tubes were incubated for forty-eight hours at 37° C.

Out of the thirty-seven food preparations which were submitted for examination, twenty-seven were stated to have contained 0.1 per cent of sodium benzoate. In one other instance sodium benzoate was employed, but the amount added was not stated. It is believed that in most if not all the instances where sodium benzoate was employed as a preservative, sterilization by heat was also practiced, but on this point I have no actual proofs.

It is noteworthy that of the twenty-seven samples of preparations preserved with the aid of sodium benzoate in 0.1 per cent concentration, twenty-two gave indications of containing small numbers of bacteria or their spores.

The features noted in regard to the growths in the fermentation tubes were specially the degree of turbidity in the bulb and closed arm of the tube, the presence or absence of a pellicle, and the presence or absence of gas. In no instance was there any gas production in a tube. In two instances a growth was observed in saccharose bouillon; once without the formation of a pellicle, once with the formation of a pellicle. In eight instances abundant growths were observed in the fermentation tubes containing lactose bouillon, in each instance without the formation of a pellicle. In none of these eight instances of bacterial growths in the lactose bouillon fermentation tubes was there any growth in the saccharose or the dextrose tubes. In seven instances abundunt growths were observed in the dextrose bouillon fermentation tubes; three times with the formation of a pellicle, four times without the formation of a pellicle. In all seven instances mentioned the dextrose tubes alone contained growths, the saccharose and lactose tubes being free from growths. In three instances, growths were observed in all three media, with the formation of a pellicle. In two instances growths were observed

in the dextrose and the lactose fermentation tubes with the formation of a pellicle, no growth occurring in the saccharose tube. In one instance a growth, with a pellicle, occurred in the dextrose and saccharose tubes but not in the lactose tube.

These observations make it clear that the addition of sodium benzoate in 0.1 per cent concentration to the varieties of food preparations in question cannot be regarded as insuring the absence of living bacteria or their spores, notwithstanding the fact that it is probable that in most of the instances sterilization by heat had also been employed. On the other hand it is to be noted that the types of organisms which grew in the fermentation tubes were not numerous. Three different organisms which were isolated were spore-formers and it is not unlikely that all of the organisms which were obtained in the fermentation tubes belong to the spore-forming class. The spores in each instance were observed to be very resistant to the action of heat. No extended effort was made to identify the organisms observed, but the available indications (size, form, motility, behavior towards Gram-stain) make it probable that most of them belong to the subtilis group.

II. Observations were also made on the action of sodium benzoate and benzoic acid, sodium hippurate and hippuric acid on pure cultures of certain types of intestinal bacteria. The procedure employed consisted of adding these various substances to plain bouillon, to dextrose bouillon, and to dextrose bouillon containing calcium carbonate. The concentrations employed in each instance were 0.05 per cent, 0.1 per cent, and 0.2 per cent. At the end of twenty-four hours and again at the end of forty-eight hours all the tubes were examined and compared with control fermentation tubes containing media to which no preservative had been added. The results were then tabulated. The organisms employed were the following: *B. coli*, *Mic. ovalis*, *Mic. albus*, *Bact. aërogenes*, *B. infantilis* (two strains, one of which was acidophilic) and a pseudo-gas bacillus. In most instances in which benzoic acid or sodium benzoate was added the growths were somewhat inhibited but the effects observed from concentrations of 0.05 and 0.1 per cent were much less pronounced than where the concentration was 0.2 per cent. In general, inhibition was more marked from the action of sodium benzoate than in

case of ketsups, where I understand it has been found necessary to use 0.2 or 0.3 per cent to protect against bacteria and yeasts.

It is possible that in semi-solid food preparations a concentration of 0.1 per cent sodium benzoate is all that is required to prevent the multiplication of ordinary microörganisms of the air. Definite knowledge of the influence of the water content of such preparations on the efficiency of the protective action of sodium benzoate is a desideratum.

III. Some observations were made on the action of sodium benzoate and benzoic acid on the mixed intestinal flora of normal individuals. The general procedure employed here was as follows: Approximately 0.1 gram of representative fecal material was suspended in sterile physiological saline solution and inoculated into fermentation tubes containing dextrose bouillon, or dextrose bouillon to which calcium carbonate had been added in excess. The concentration of the preservatives varied from 0.05 to 0.2 per cent. Control experiments were made with corresponding media containing no preservative. Observations were made upon the turbidity of the growths, on the gas production and on the character of the sediments. The tubes were incubated at 37° C. and observations were made and recorded at the end of twenty-four and forty-eight hours, respectively. The following results were obtained. Sodium benzoate in 0.1 per cent concentration and benzoic acid in 0.05 per cent concentration, in dextrose bouillon, were found to inhibit the formation of gas. Sometimes after twenty-four hours a few millimeters of gas were observed in the tubes containing sodium benzoate, and both in the case of the benzoate and the free acid moderate numbers of Gram-negative bacilli referable morphologically to the *B. coli* group were observed. A few coccal forms and some Gram-positive rods were also observed. Organisms of the bifidus type were very seldom observed. A few bacteria of acidophilic character were generally observed, but organisms of the latter type were frequently noted in fewer numbers than in the sediments of the control fermentation tubes. When the preservatives were used in higher concentrations, that is 0.2 per cent sodium benzoate and 0.1 and 0.2 per cent benzoic acid, Gram-negative forms referable to the *B. coli* type were frequently less numerous than in the control tubes. On the other hand the

coccal forms usually did not show such inhibition but, on the contrary, sometimes exhibited a marked overgrowth, especially in the case of the Gram-positive forms. Sometimes there was a slight increase in the numbers of Gram-negative bacilli and cocci, but this was an inconstant modification.

In dextrose bouillon to which calcium carbonate had been added gas formation was distinctly increased. The amount of gas was usually greater than in the control fermentation tubes containing calcium carbonate. The Gram-negative forms of the *B. coli* type and some other Gram-negative forms accumulated more abundantly in the fermentation tubes than in dextrose bouillon fermentation tubes containing preservative but no calcium carbonate.

It thus appears that sodium benzoate and benzoic acid in the concentrations employed in these experiments have the power of diminishing the gas production in fermentation tubes and at the same time modifying somewhat the character of the fermentation tube sediments. The chief modification appears to be an inhibition in the growth of organisms of the *B. coli* type and a relative increase in coccal forms of bacteria. In fermentation tubes containing calcium carbonate, the Gram-negative colon-like organisms were not inhibited as in the case of tubes containing no calcium carbonate.

In this connection may be mentioned the action of sodium benzoate on the gas-forming properties of human mixed intestinal bacteria, as reported by me in the research from my laboratory published in Bulletin No. 88 of the United States Department of Agriculture. It was observed that the fecal flora of persons taking several grams of sodium benzoate daily no longer produced as much gas as previously (when smaller quantities of sodium benzoate had been taken) in dextrose bouillon. There appeared to be no doubt as to the relation between the taking of the benzoate and the diminished gas production. The preparatory period on small doses of sodium benzoate does not seem to be necessary for the development of this effect of larger doses. One of our subjects was especially studied with reference to this point. The administration of four grams daily of sodium benzoate for several days was soon followed by a diminished power of gas production in each instance in a series of such trials.

It should also be noted that the periods of diminished gas production appeared to correspond to a change in the nature of the fermentation tube sediments, a change characterized by the appearance of an increased prominence of coccal forms of bacteria. The basis of this statement is as follows: The fermentation tube sediments were examined by me without any knowledge of the position of these preparations in the series to which they belonged. Despite this random examination of the fermentation tube sediments, it was possible to form a fairly accurate judgment in the case of each subject of the position of the slide in the series with respect to the period of low gas production. This judgment was also obtained through guidance by the relative overgrowth of coccal forms. In the slides in which such overgrowth was most marked a correspondence with a period of low gas formation was assumed and this assumption was in general justified.

It should be clearly understood that the increase in coccal forms observed in the fermentation tube sediments was not associated with any demonstrable increase of coccal forms in the fecal smears made direct from the feces.

I am unable to offer any satisfactory explanation of the depression of gas production in fermentation tubes, following the use of considerable doses of sodium benzoate. It seems probable that the phenomenon can be reproduced in monkeys, and if this is the case an explanation of this peculiar effect of the sodium benzoate might, perhaps, be obtained from a study of the bacteria and of the chemical conditions at various levels of the digestive tract. Perhaps the direct action of sodium benzoate on the intestinal bacteria in the upper part of the digestive tract is answerable for the poor gas production.

Dr. Dakin was so good as to make some observations for me on the action of sodium benzoate upon the activity of brewer's yeast in a beerwort medium. The medium originally contained 7.14 per cent of glucose (or reducing sugars calculated as glucose). After fermentation for forty-eight hours at body temperature the medium contained 1.4 per cent of glucose. A portion of the medium to which 0.15 per cent sodium benzoate had been added contained 4.33 per cent glucose, thus showing a considerable inhibiting action on the part of the benzoate. The medium fermented without benzoate contained 5.50 per cent alcohol at

the end of the experiment. The medium fermented in the presence of sodium benzoate contained 3.14 per cent alcohol. The volatile acids (calculated as acetic) were 0.01 per cent after fermentation without benzoate, and 0.02 per cent[1] after fermentation in the presence of sodium benzoate. The non-volatile acids (calculated as succinic acid) amounted to 0.05 per cent in the medium fermented without benzoate present, and to 0.02 per cent in the medium fermented in the presence of benzoate.

These figures thus indicate that sodium benzoate in concentration of 0.15 per cent inhibits markedly the fermentative activity of the yeast plant in a favorable medium, while permitting a considerable formation of alcohol. It was thought that perhaps the diminution in gas production caused by the presence of sodium benzoate might be associated with some increase in the non-volatile acids, especially lactic and succinic but this experiment gives no support for this possibility.

CONCLUSIONS.

1. Commercial food preparations to which sodium benzoate had been added in concentration of 0.1 per cent were found in most instances to contain small numbers of bacteria, chiefly of the spore-bearing kind.

2. Sodium benzoate in dextrose bouillon, in concentration of 0.1 per cent only slightly or moderately inhibits *B. coli* and other intestinal bacteria. Gas-production may, however, be considerably inhibited.

3. When mixed fecal bacteria from normal adults (on a mixed diet) are introduced into dextrose bouillon fermentation tubes containing 0.1 per cent or 0.2 per cent sodium benzoate, the bacteria are unequally inhibited. In general the organisms of the *B. coli* group appeared to be more inhibited than the coccal forms of bacteria.

I desire to acknowledge valuable aid given me by Mr. A. I. Kendall, lately Fellow of the Rockefeller Institute, in the bacteriological work on which this paper is founded.

[1] After removal of benzoic acid by chloroform extraction.

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AN OBSERVATION ON THE FATE OF B. BULGARICUS (IN BACILLAC) IN THE DIGESTIVE TRACT OF A MONKEY

(PLATES I–III)

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The incentive to make the observations here recorded came from certain problems suggested by the rapidly extending use of artificially soured milk which is following closely upon the publication of Metchnikoff's highly speculative volume, entitled "The Prolongation of Life." Really decisive experiments bearing upon the value of what may be termed "sour milk prophylaxis" are not numerous and even Scriptural quotations have been invoked to lend color to, and enhance the meager supply of literature. One of us[1] has studied the effect of introducing large numbers of *B. coli*, *B. proteus vulgaris* and *B. acidi lactici* into the intestinal tract of dogs to determine their action upon indol production. Living cultures of *B. coli* and *B. proteus*, for example, caused an increase of indican and of ethereal sulphates in the urine.[2] Killed cultures prepared in the same manner gave little or no increase in these putrefactive constituents.

[1] C. A. Herter: On Certain Relations Between Bacterial Activity in the Intestinal Tract and the Indican of the Urine. *Brit. Med. Jour.*, 1897, ii, p. 1847.

[2] Precautions were taken to prevent the introduction of putrefactive products derived from the media upon which the bacteria were grown. The organisms were cultivated upon slanted agar (removed by careful washing with salt solution, avoiding the inclusion of any portion of the agar) and then injected.

Lactic acid bacteria injected directly into the small intestine in similarly conducted experiments showed a tendency to cause a reduction in the output of indican and ethereal sulphates.

Following similar lines, Metchnikoff directed the performance of experiments which lead him to believe that lactic acid-producing bacilli once successfully established in the intestinal tract decrease or even prevent the multiplication of putrefactive organisms. This inhibiting effect he attributes to the lactic acid which is produced by microörganisms introduced in these experiments.

Where putrefactive disturbances are already present the lactic acid bacilli appear to reenforce the enfeebled action of the normal intestinal lactic acid bacilli and increase the amount of acid produced where it is insufficient, or reintroduce it where it is absent, thus assisting the host to throw off the "wild races" of bacteria that may have become habituated to the intestinal tract. This, at least, is the assumption.

Not all lactic acid-producing bacteria are suitable for this purpose and the choice of a culture should be based upon the following criteria: First, the organisms should be able immediately or in time to become habituated to the intestinal tract; second, they should produce no toxins or putrefactive substances or other injurious products, detrimental to the host; third, they should be able to make sufficient lactic acid to accomplish the purpose for which they are introduced.

The organism which Metchnikoff selected for his researches was originally obtained from the Bulgarian ferment called "Yougourt" and the bacillus which apparently is the most potent lactic acid producer in this ferment has been named *B. bulgaricus*.[1] This organism, as we have studied it, is rather long (1 × 4–6 microns), large, with rounded ends, growing singly or in pairs, rarely in chains. It stains well with the ordinary anilin dyes particularly in young cultures, and is Gram-positive. In old milk cultures some of the rods may be Gram-negative while others present a punctate appearance, due apparently to the concentration of protoplasm in certain portions of the cell which are Gram-positive, and suggest Ernst-Babes granules, the remaining por-

[1] Cohendy: *Compt. rend. de la soc. de biol.*, lx, 1906.

tions undergoing rarefication of protoplasm and becoming Gram-negative. On media containing no carbohydrates there is no growth. On dextrose or lactose agar the growth is slight and usually appears as small, stellate colonies rarely exceeding one to one and one-half millimeters in diameter. In stab cultures on the same media a slight growth appears after three to five days but always remains limited to the line of inoculation. In dextrose and lactose bouillon there is usually a feeble growth after several days, appearing as a fine sediment visible only after agitating the tube. In milk cultures, on the contrary, the growth is very vigorous, resulting in a rather finely flocculent coagulum with a minimal separation of fluid. In this medium considerable amounts of lactic acid are formed. Bertrand and Weissweiller[1] studied the chemical action of *B. bulgaricus* on milk and found that a slight amount (usually less than 10 per cent), of the casein is peptonized and apparently utilized as food by the bacterial cells. A small amount of the fat is saponified while practically all of the lactose is changed to dextro- and lævo-lactic acid, the dextro variety predominating. Twenty-five grams per liter of lactic acid are easily formed and at the same time small amounts of acetic, formic and succinic acids are produced, usually not more than half a gram per liter. Inoculations into milk are active, even after fourteen days, although at the end of three weeks the bacilli are usually dead. In our experience the amount of lactic acid produced by *B. bulgaricus* is sufficient at the end of forty-eight hours to render the milk unpleasant to the taste, particularly if previous to inoculation the culture has been frequently transplanted, so that the organisms are in an active vegetative state. Soured milk prepared according to Metchnikoff's directions is said to contain about ten grams of lactic acid per liter.[2]

Several investigations have been made by various observers to determine the effects of the Bulgarian bacillus upon the intestinal flora and various putrefactive products. Cohendy,[3] experimenting upon himself, found that pure cultures of lactic acid bacill had a tendency to reduce intestinal putrefaction and that the

[1] *Ann. de l'inst. Past.*, xx, pp. 977–990, 1906.
[2] Metchnikoff: *The Prolongation of Life*, p. 180.
[3] *Loc. cit.*

organisms could be recovered from the feces without difficulty several weeks after the experiment was stopped. He took *B. bulgaricus* for about two and one-half months. Pochon[1] consumed cultures of lactic acid bacilli in milk and noted the diminution of indol and phenol in his feces. Leva[2] investigated the effect of Lactobacilline, milk, and milk plus Lactobacilline upon the excretion of ethereal sulphates, volatile fatty acids, aromatic oxyacids, phenol and indican. The experiment was divided into four periods: (1) a uniform daily diet; (2) diet + Lactobacilline;[3] (3) diet + Lactobacilline + one liter of milk; (4) diet + one liter of milk. His conclusions are as follows:

(1) The excretion of ethereal sulphates during the experiment was practically unchanged.

(2) The excretion of volatile fatty acids with Lactobacilline alone, or milk alone, as well as with Lactobacilline and milk combined, showed a considerable decrease.

(3) The excretion of aromatic oxyacids and hippuric acid was uninfluenced by milk, decreased distinctly in amount with Lactobacilline, decreased greatly with Lactobacilline + milk.

(4) The phenol excretion decreased somewhat under the influence of Lactobacilline alone, as well as with milk alone; there was a much greater decrease with a combination of Lactobacilline + milk.

(5) The indican excretion was very slight at the beginning of the experiment (too small an amount to measure accurately) and remained practically unchanged throughout the entire period.

Belonowsky[4] studied the influence of these organisms upon the intestinal flora of mice. His method was to contaminate the food (usually grain previously sterilized by heat) with *B. bulgaricus*, allowing the animals to eat sufficient quantities to make certain that the organisms were actually introduced in large numbers. His results may be summarized as follows: First, the Bulgarian

[1] Cited by Combe: *L'autointoxication intestinale*, Paris, 1906.

[2] Leva, J.: Zur Beurteilung der Wirkung des Lactobacillins und der Yoghurthmilch, *Berl. klin. Wochenschr.*, xlv, pp. 922–924, 1908.

[3] The Lactobacilline was obtained from "Le Ferment" Company of Paris. Leva's observation that a yeast was present in the Lactobacilline agrees with our own observation on this point. (See Fig. I.)

[4] *Ann. de l'inst. Past.*, xxi, p. 991, 1907.

ferment modifies the normal intestinal flora of mice by a general alteration in their character and by elimination of putrefactive forms. There is a diminution in the total number of bacteria as well as a lessened virulence of the feces when these are introduced intraperitoneally or subcutaneously into other animals. Second, the action is not attributable to the formation of lactic acid alone, but also to certain products inhibitory in nature, formed by the bacilli themselves. Third, the organisms become more or less established in the intestine about the tenth day in adult mice and persist without further reinoculation for a considerable but variable interval of time. Fourth, the cultures seem to have exerted a beneficial action upon the mice, particularly on those infected with the organisms of mouse typhus; in this case the results are due exclusively to the lactic acid.

From these investigations it would appear that many of the animals fed upon grain contaminated with the Bulgarian ferment gained in weight, that the feces contained fewer organisms capable of growing upon ordinary culture media, that putrefactive organisms tended to disappear and that this beneficial action was due in part to the lactic acid, in part to the products of metabolism of the bacteria themselves. The fact that relatively few of the Bulgarian bacilli are microscopically discernible in the feces raises the question, In what portion of the intestinal tract do these bacilli find their most favorable environment? If they occur in the upper (duodenal or jejunal) regions of the small intestine and only a few gain a foothold in the large intestine (usually considered the chief site of putrefaction) the organisms must act from a distance and their products, theoretically at least, must be less effective than if they were generated at the focus of infection. No experiments so far have been recorded which answer this question and the present investigation has approached the problem from this point of view. For our work, which was undertaken specifically to study the distribution of *B. bulgaricus* in the intestinal tract, the preparation called *Bacillac* was employed. This is said to be made according to Metchnikoff's personal directions, cultures of the organism described above (*B. bulgaricus*) being employed for this purpose. This organism is stated to have been isolated by Metchnikoff. The *Bacillac* is obtainable in pint bottles and contains a moderately large, Gram-positive

bacillus and (by accident or design) a large, oval, Gram-po
yeast as well (see Fig. 1). The bacillus isolated from spec
of Bacillac grows slowly upon ordinary dextrose and lactose
very poorly in corresponding bouillon media, but luxurian
milk. It produces in the latter medium a soft coagulum
after standing for a few days becomes massed into more o
permanent lumps with a moderate separation of fluid.
acidity increases rapidly and after forty-eight hours bec
decidedly unpleasant to the taste.

Partly because of its rapid growth, but chiefly because c
considerable amount of acid which this bacillus produces,
easy to obtain cultures of the organism grown in milk at 3
even if it be originally associated with other organisms. C
sub-culturing gives a differential enrichment of the Metchn
bacillus, so that one may obtain it in pure culture, as ma
demonstrated by plating on slightly acid Bierwort agar. I
present investigation this method of enrichment has been
successfully for the isolation of the organism from the n
intestinal flora.

For experimental purposes a moderate sized Rhesus mo
was used. The animal received daily half a liter of sweet
for a period of three days. The feces were examined for
acid[1] as well as for organisms resembling the Metchnikoff ba

[1] Fletcher and Hopkins: *Journ. of Physiol.*, xxxv, pp. 247–309, 19
Reagents:

(1) Very dilute alcoholic solution of thiophene (10 to 25
in 100 cc.
(2) Saturated aqueous solution of copper sulphate.
(3) Concentrated sulphuric acid.

Procedure: 5 cc. strong sulphuric acid, 1 drop copper sulphate
few drops of the suspected mixture are well shaken, then heated for
minutes in a water-bath in a test tube. Cool the solution, add 2 to 3
thiophene solution, replace in water-bath and again heat, watching
stantly. Lactic acid rapidly and characteristically gives a bright ch
red color under these conditions. The lactic acid must be as nearl
from water and organic matter as possible (malic acid and probably
oxy-acids give the reaction).

The lactic acid may be employed as an alcoholic solution or as a s
residue. Acetaldehyde and glyoxylic acid give color reactions with
phene and sulphuric acid, but the copper sulphate used as above des

either culturally or morphologically. In no instance was lactic acid found.

The experiment with *Bacillac* was carried out as follows. The amount of soured milk given was the same as in the control observations, namely, five hundred cubic centimeters daily. At the end of the second day, chemical and bacteriological examinations were commenced, but until the sixth day no lactic acid bacilli were isolated nor could lactic acid be detected in the animal's feces. Two days later the first positive test for lactic acid was obtained, using the thiophene reaction of Fletcher and Hopkins. The feces were slightly but distinctly acid at this time—more acid than upon previous occasions. It was difficult to obtain a satisfactory test for lactic acid from the feces owing to the presence of a brownish-yellow coloring matter soluble in ether. But it was possible by removing the ether through evaporation, taking up the oily residue in water, boiling with animal charcoal, filtering, washing, and again evaporating to a syrup to obtain a slightly yellow solution which gave a good reaction with the thiophene. Controls made from normal feces of the same animal (free from lactic acid), which were treated in the same manner, but to which were added known minute amounts of lactic acid, gave in every instance the same color reaction. After fourteen days the monkey was given the usual meal of five hundred cubic centimeters of *Bacillac*. Then, after allowing three and one-half hours for digestion (the whole portion of milk having been consumed at this time) the animal was killed by chloroform and examined. Samples taken from the stomach and from various levels of the small and large intestines were removed with appropriate precautions for microscopical, bacteriological and chemical examination. The material for chemical examination was placed in ether slightly but distinctly acidified with sulphuric acid. The bacterial material was inoculated into milk and fermentation tubes containing dextrose, lactose and saccharose bouillon. The specimens for microscopical study were smeared on slides and stained by the Gram method for identification of forms resem-

them. If ether is employed for extracting lactic acid, it should be first washed with water to remove aldehyde bodies. The color produced by lactic acid is transitory unless the tube be cooled immediately after its appearance.

bling morphologically the acid bacillus fed. The animal was in good health before and during the experiment. At autopsy about one hundred cubic centimeters of partially digested milk were found in the stomach. The small intestine contained a moderate amount of semi-fluid, yellowish, gelatinous substance. At the ileo-cæcal valve the contents were more abundant and about the consistence of thick paste. The color was a deeper brown. The color and consistence increased progressively to the anus, where the feces were solid and fairly dark. The mucous membrane throughout the gastro-intestinal tract appeared normal, although the reaction of the contents from the stomach to the anus was distinctly acid to litmus.

The Bulgarian bacilli were present and easily demonstrated by smears and by cultures in relatively large numbers in the stomach contents but associated with yeasts. In some instances, particularly where the milk was obviously undergoing digestion, the organisms showed undoubted signs of degeneration. The staining was very irregular and faint in such bacilli, whereas the yeasts, so far as could be determined by microscopical examination, showed no such changes. In the duodenum and ileum the Bulgarian organisms were encountered in almost pure culture, although inoculations into fermentation tubes showed a few gas-forming bacilli of the colon type. In the region of the ileo-cæcal valve there was a rather abrupt change in the nature of the bacterial smears. Not only was the amount of fecal material greater but the character of the microörganisms was different. *B. bulgaricus* ceased to be the dominant organism, although it was still present in moderate numbers. Gas-forming bacteria were, on the other hand, increased enormously. Gram-positive rods and cocci were also present. No attempt was made to identify the latter. From the ileo-cæcal valve, progressively down the large intestine towards the anus, the number of the Bulgarian bacilli decreased while the number of other bacteria increased, until at the rectum there were very few Metchnikoff bacilli but enormous numbers of bacteria of the colon type and many Gram-positive rods.

Lactic acid was demonstrated throughout the gastro-intestinal tract as well as in the feces. Although no attempt was made to determine quantitatively the amount of acid at any level of the

intestine, the results indicated that much less lactic acid was present in the large intestine than in the stomach and small intestine. This diminution (shown by the decidedly lessened color developed by the thiophene, using approximately equal amounts of intestinal contents) began rather abruptly at the region of the ileo-cæcal valve, and progressively increased to the anus. This phenomenon was particularly marked in the lower portions of the large intestine, where the contents were more desiccated. The amount of material obtained from this region was greater than was the case in higher levels, while at the same time the volume of lactic acid was decidedly less, although the ether extraction was prolonged. This coincides with the relatively smaller number of lactic acid bacilli found.

CONCLUSIONS.

(1) By feeding a Rhesus monkey for two weeks exclusively on milk fermented with *B. bulgaricus* (but containing also some yeasts) it was possible to maintain an acid reaction throughout the digestive tract. The acid reaction was more pronounced above the ileo-cæcal region than at this region or below it. The acidity decreased progressively from the ileo-cæcal region to the anus. Lactic acid was detectable at every point in the digestive tract that was tested, the reaction growing less marked below the ileo-cæcal region.

(2) Exclusive feeding for two weeks with milk fermented with *B. bulgaricus* failed to establish the predominance of this organism in the ileo-cæcal region or in the large intestine. In the latter situation the number of bacilli of this type was relatively small and decreased towards the anus. Thus in the regions characterized by most active putrefaction the lactic acid bacilli failed to establish themselves in relatively large numbers.

DESCRIPTION OF THE PLATES.

Fig. I. Smear from stomach contents of monkey, three and one-half hours after feeding *Bacillac*. Practically pure culture of *B. bulgaricus* and few yeast cells. Bacteria show "punctate" staining due, apparently, to combined effect of partial digestion and excessive acidity.

Fig. II. Contents of small intestine in the region of the duodenum. The Bulgarian bacilli predominate. A few punctate forms are seen. The bacteria are multiplying at this point. The normal appearance of the bacteria is in accordance with this observation.

Fig III. Contents of the small intestine near the ileo-cæcal valve. Uniformly staining Bulgarian bacteria are present, but not in predominating numbers as in Fig. II. Gram-negative forms begin to predominate.

Fig. IV. Contents of the large intestine about two feet from the ileo-cæcal valve. A few typical Bulgarian bacilli still persist. One of these shows the punctate staining seen in the stomach. Yeast cells are also present. Gram-negative bacilli are the dominant forms.

Fig. V. Feces of monkey. Bulgarian bacilli have practically disappeared. They were, however, readily demonstrated by the milk-enrichment method described in the text.

Fig. VI. Fermentation sediment obtained from a lactose fermentation-tube inoculated with intestinal contents obtained from the level of the ileo-cæcal valve (cf. Fig. III). No Bulgarian bacilli are present in this sediment, although they were readily demonstrated in the smear made at this level as well as culturally in milk. (This figure is introduced to show that the Bulgarian bacilli do not grow in fermentation media.)

The slides from which these plates are reproduced were stained by Gram's method. Magnification, 1000 diameters. Zeiss 2 mm. homo. imm. apochromatic lens.

Our thanks are due to Dr. Leaming of the Rockefeller Institute for Medical Research for the above photographs.

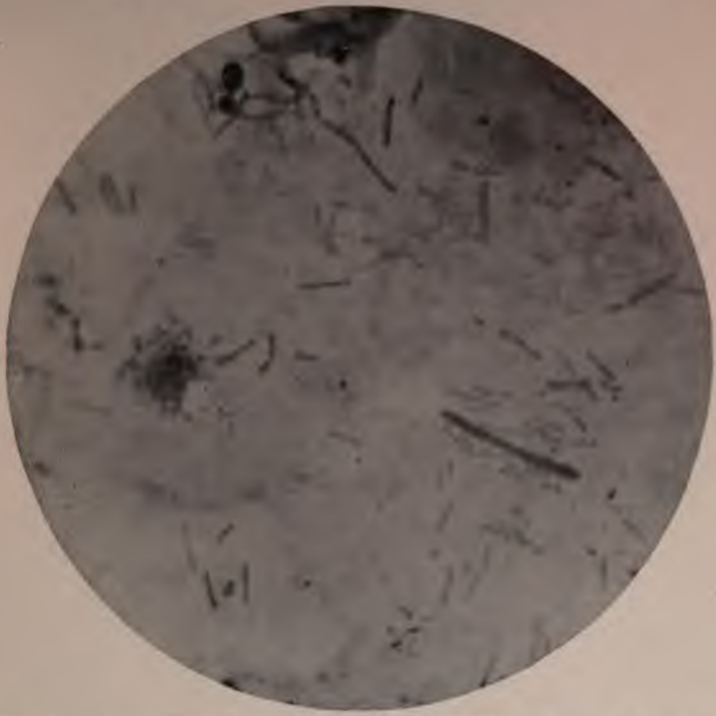

Fig. I. Smear from stomach contents of monkey, showing great predominance of *B. Bulgaricus* and a few yeast cells.

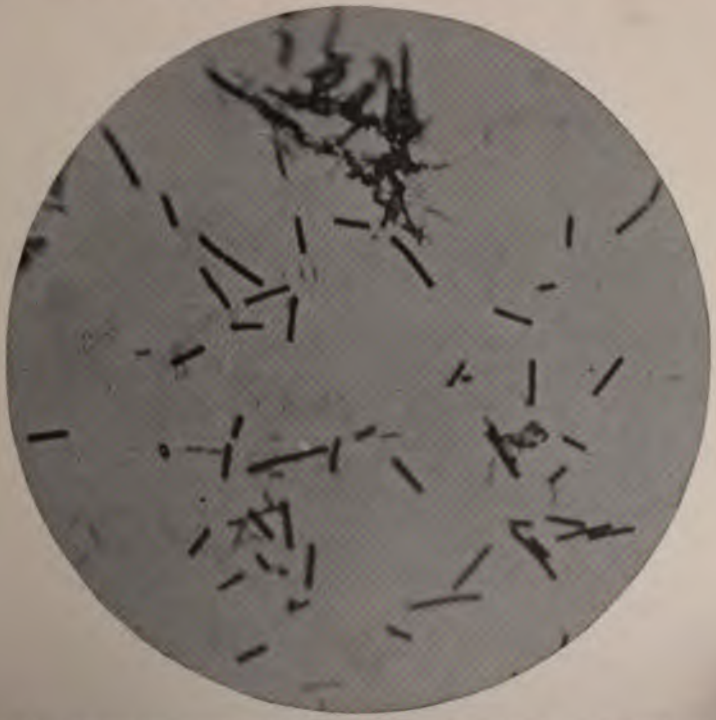

Fig. II. Smear from contents of duodenum: *B. Bulgaricus* predominating.

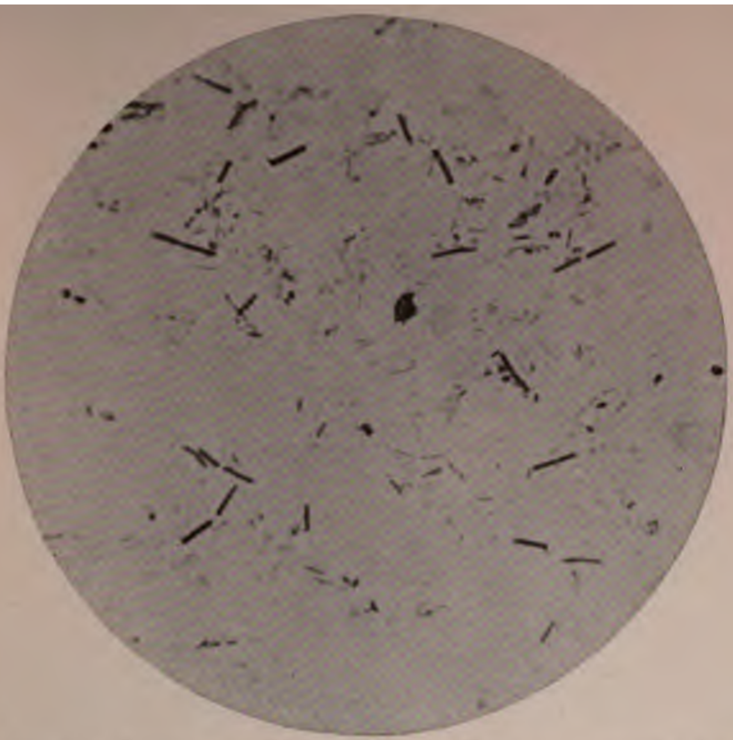

Fig. III. Smear from small intestine: *B. Bulgaricus* prominent but not predominating.

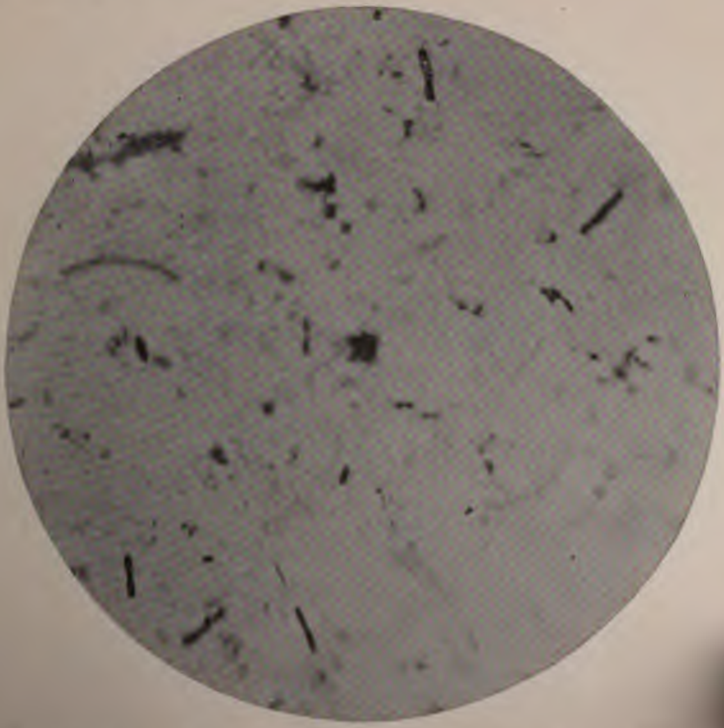

Fig. IV. *Smear from large intestine:* B. Bulgaricus much less prom*inent than in regions above.*

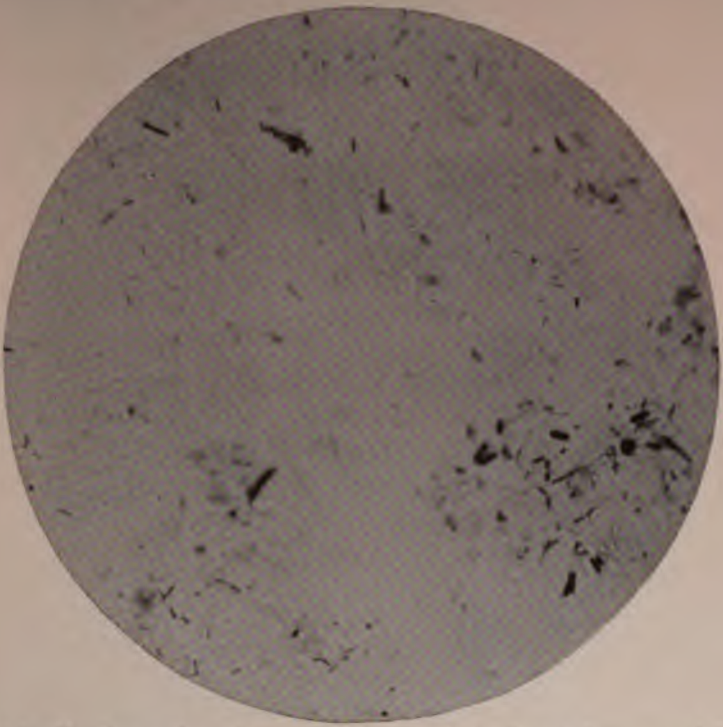

Fig. V. Smear from feces: *B. Bulgaricus* has almost disappeared.

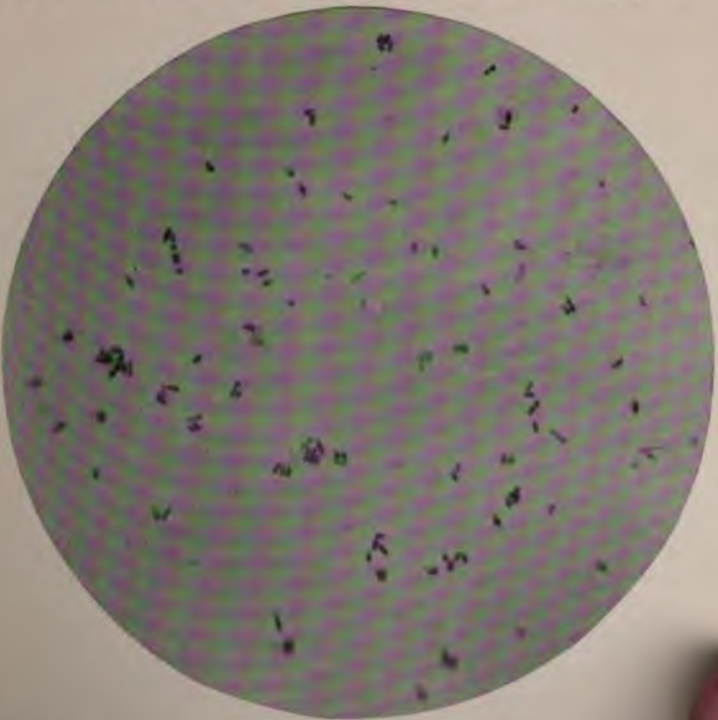

Fig. VI. Smear from fermentation-tube sediment from lactose fer[illegible]tation-tube inoculated with intestinal contents from the level o[illegible] ileo-cæcal valve.

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THE USE OF THE FERMENTATION TUBE IN INTESTINAL BACTERIOLOGY

BY

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THE USE OF THE FERMENTATION TUBE IN INTESTINAL BACTERIOLOGY.

By C. A. HERTER, M.D., and A. I. KENDALL, Ph.D.

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During the progress of a series of investigations bearing upon the bacterial flora of the intestinal tract of infants and adults in health and disease, the writers have obtained valuable information from the routine use of the fermentation tube, by using methods similar to those of Dr. Theobald Smith,[1] who has repeatedly called attention to the fundamental importance of this apparatus in the study of fermentative bacteria. His researches have shown conclusively that the gas volume, gas ratio (proportion of gas soluble in caustic alkali to the insoluble portion) and the length of time necessary to produce the maximum amount of gas are characteristics of prime importance in the study of this group of organisms. Furthermore, by the simple addition of bits of fresh sterile animal tissue[2] he has succeeded in cultivating in these tubes a number of anaërobes which would not grow under ordinary conditions.[3] Finally, by introducing the use of milk in

[1] Das Gährungskölbchen in der Bakteriologie, *Centralbl. f. Bakt.*, vii, pp. 502–506, 1890; Einige Bemerkungen über Säure- und Alkalibildung bei Bakterien, *Ibid.*, viii, p. 389, 1890; The Fermentation Tube, etc., *Wilder Quarter-Century Book*, pp. 187–234, 1893.

[2] *Centralbl. f. Bakt.*, vii, pp. 502–506, 1890.

[3] There seems to be considerable question about the priority of this procedure. According to Marino (Méthod pour isoler les anaërobies, *Ann. de l'inst. Pasteur*, xxi, p. 1005, 1907) the credit belongs to Duenschmann (Étude experimentale sur le charbon symptomatique et ses relations avec l'oedème malin, *Ann. de l'inst. Pasteur*, p. 482, 1895) who mentions the fact that Roux, under whose direction this work was carried out, had previously used serum (beef) to cultivate certain anaërobes. As a matter of fact Theobald Smith (Das Gährungskölbchen in der Bakteriologie, *Centralbl. f. Bakt.*, vii, p. 502, 1890) very distinctly mentions the fact that sterile animal tissue may be employed to advantage in the cultivation of certain obligate anaërobes and he actually employed fermentation tubes enriched in this manner at that time.

the fermentation tube, he has added much to the value of this medium for bacterial research.[1]

Smith's published results have been obtained chiefly with pure cultures. Herter and Ward[2] have studied the gas volume with fermentation tubes inoculated with mixed intestinal flora and Herter[3] has studied the sediments derived from the inoculation of such tubes.

TECHNIQUE.

(1) *Preparation of sample.* It is necessary at the start to have fresh specimens of stools for investigation—samples that have stood for some time or have been freely exposed to the air or to high temperatures are liable to change rapidly in their bacterial composition. Nonspore-forming anaërobes may rapidly succumb while, coincidently, hardy forms multiply until they exist in abnormally large numbers, thus influencing very markedly the bacterial aspect of the result.

It is necessary in this particular line of investigation for several reasons to inoculate appropriate amounts of fecal material; the portion added must not be too great or the acids and other antagonistic products formed by the more rapidly growing facultative organisms will seriously inhibit the growth of the more strictly parasitic types; too small an amount, on the other hand, may fail to furnish a representative growth of significant microörganisms. The less numerous but perhaps extremely important forms may, without these precautions, be overlooked and missed entirely.

For routine purposes one gram of feces thoroughly emulsified in ten cubic centimeters of physiological saline solution is an

[1] Milk intended for the fermentation tube must be sterilized at least four successive times at appropriate intervals to insure the absence of resistant spore-forming bacteria which ordinarily escape observation. After sterilization and before inoculation milk fermentation tubes must be incubated several days at body temperature to test their sterility. See Smith, Brown and Walker: The Fermentation Tube in the Study of Anaërobic Bacteria with Special Reference to Gas Production and the Use of Milk as a Culture Medium, *Journ. Med. Research*, xiv, pp. 193–206, 1905.

[2] This *Journal*, i, p. 415, 1906.

[3] *The Common Bacterial Infections of the Digestive Tract*, 1907.

excellent dilution. Probably all types present that are capable of development in fecal media are thus represented.[1] If mucus is present in the stool it should be washed in sterile water and inoculated separately. One cubic centimeter of the suspension is placed in each tube.

Certain forms, as for example, the *B. bulgaricus* described by Metchnikoff, and many alkali-producing bacteria, will not grow in the ordinary fermentation media but usually develop rapidly in milk fermentation tubes.

The period of incubation is a very important factor. Experience has shown that during the first eighteen to twenty hours (rarely longer than this) the majority of the vegetative cells will be at their maximum growth; after this time, owing partly to antagonism, partly perhaps to the fact that the nutrient material that was carried over with the suspension is exhausted, many forms die out, while the more saprophytic organisms increase enormously.

The actual gas volume is rather less at twenty hours than at subsequent periods as a rule, but the relative value of this feature is at the maximum. The bacteria derived from ordinary stools, particularly of the colon type, tend to attain a more or less constant gas volume after forty-eight hours.

In plain bouillon without the addition of carbohydrates (particularly with media made from meat juice instead of meat extract as a basis, gas is sometimes liberated after acidification of the culture with hydrochloric acid, although no gas was present during the incubation period. Also the addition of cystin to the medium tends to increase the gas volume. This gas is hydrogen sulphide, and its total amount may be estimated with a considerable degree of accuracy through the absorption effected by the addition of a soluble salt of a heavy metal.

The gas ratio is not an especially important characteristic in mixed fecal flora, much less so in fact than is the case with pure cultures. Frequently the volume is too small to measure, and

[1] The emulsion should be rapidly prepared and inoculated. Too long an exposure in the relatively aërobic saline solution may, and frequently does, eliminate many vegetative and nonspore-forming anaërobic forms, particularly those of infant flora, while the more vigorous aërobic bacteria increase rapidly.

dition but fifteen to twenty millimeters represents fairly the average. The amount furthermore depends upon the relative proportion of *B. bifidus* present; if the latter organism be abundant, less gas is formed, because this species produces sufficient acid to inhibit the growth of the ordinary gas-formers.

Diarrhœal stools vary in their gas production. In those cases where large numbers of cocci are brought down from higher levels of the intestine, relatively small gas volumes are the rule, while in similar movements associated with large numbers of colon bacilli, or with organisms of the lactis aërogenes type, a much greater production takes place.

Mention has already been made of the fact that the fermentation tube is a particularly simple and efficient apparatus for cultivating anaërobic intestinal bacteria. Of these organisms a few will not grow in pure culture under the same conditions, although they usually thrive symbiotically with facultative anaërobes.

There can be no doubt that certain substances, particularly favorable for the growth of these more or less strictly parasitic forms are carried into the fermentation tube as a part of the fecal suspension, and during the first eighteen or twenty hours furnish a suitable pabulum for their growth—material, furthermore, which is not present in the fermentation tube as it is ordinarily made up. It is extremely probable that their growth is further aided by the presence of more readily growing bacteria which frequently render the tubes extremely anaërobic by the removal of the last traces of dissolved oxygen.

In the fermentation tube every transition from almost complete anaërobiosis to aërobiosis obtains and it is possible for bacteria to find almost any tension of oxygen from more or less complete saturation in the bulb to practically its entire absence in the closed arm.

With such favorable conditions—proper food supply and gaseous environment—the growths are very varied and in a measure representative of the organisms originally present in the feces. This fact is best appreciated after one examines the sediments, particularly those stained by Gram's method followed by the counter stain mentioned above.

The organisms are as a rule much more characteristic morphologically in the deposit at the bottom of the closed arm than is the

case in the feces from which they were derived, because the majority are in what may be termed the "active" vegetative stage. Bacteria in this condition are larger and more nearly typical than under conditions where they have become attenuated and degenerate in their morphology, as frequently happens in the case of constipated stools. The staining reactions also are much sharper and more distinctive at this period.

Perhaps the most striking example of the differentiation one may ordinarily meet with in a sediment from a fermentation tube is that shown by a common bacterium in infants' stools called by Tissier, its discoverer, *B. bifidus communis*. This organism is an anaërobic Gram-positive bacillus, frequently occurring with rather pointed ends in normal infants' stools; not readily recognized and not especially characteristic. Furthermore it is not an easy organism to cultivate in ordinary media. In fermentation tubes, however, it grows rapidly and at the end of eighteen hours shows the peculiarly striking bifid ends to which it owes its name. This fact, judging from the literature published upon the subject so far, has hitherto been unrecognized, but it appears to be characteristic, of great constancy, and a unique example of the value of such examinations. This organism will not grow, or at least only slightly, in fermentation media in pure culture. If, however, one adds a bit of sterile animal tissue, or inoculates directly from a stool, together with other bacteria, the growth is marked.

It is advisable, and frequently necessary, to use fermentation tubes containing plain bouillon instead of the regular fermentation media. Certain bacteria will not grow well where fermentable sugars are present, while others are rapidly eliminated as the medium becomes acid. *B. putrificus* is a good example of such an organism. Sediments derived from plain bouillon in fermentation tubes, particularly those which are rendered more suitable by the addition of sterile animal tissue, frequently show anaërobic growths that would not be included in carbohydrate solutions.

One point in connection with the fermentation tubes deserves special mention—gas volumes are frequently variable with the same individual and it is necessary to cover considerable periods of time before assigning a special value to this factor in individual cases or attaching much importance to deviations from the average.

Among the organisms ordinarily met with in the feces, a few of the more important may be mentioned:

(1) *Bact. Welchii*, a thick, rather large, strongly Gram-positive bacillus. In large numbers they give rise to a considerable augmentation of the normal gas volume, so that the amount is frequently twice the normal.

An organism described by Herter[1] resembles the gas bacillus morphologically but does not form gas.

(2) *B. coli*. Short bacilli, about one micron in diameter, Gram-negative. These organisms usually determine the gas volume and it is chiefly to their action that the normal gas volume is due.

(3) *B. lactis aërogenes*, somewhat more oval than the colon bacillus and like that organism, Gram-negative. This form is not particularly common in the stools of adults[2] but is present usually in the excreta of bottle-fed infants and tends to increase the gas volume, if numerous.

(4) Coccal forms, usually Gram-positive. These bacteria produce as a rule considerable amounts of acid but no gas, and inhibit to a considerable degree the fermentative action of the above mentioned forms.

(5) A Gram-positive bacillus with bifid ends (*B. bifidus*). It is very common in the stools of breast-fed infants. When this organism is present in numbers, the amount of gas is usually considerably reduced. Its inhibitory action is due, as is the case with the coccal forms, to its excessive acid production.

In several instances, *B. bifidus* has been isolated from mucus, while the remainder of the stool was almost devoid of these forms.

The full significance of the fermentation-tube sediments cannot be regarded as completely worked out. It is a striking peculiarity of the growths in the sediments that they frequently do not show a multiplication of microörganisms closely representative of the varieties which are seen in the Gram-stained fields of the feces. This failure in correspondence between the characters of the dominant organisms in the fermentation tubes on the one

[1] *Loc. cit.*

[2] MacConkey (Lactose-fermenting Bacteria in Feces, *Journ. of Hygiene*, 1905, pp. 333–379) found it in 4 out of 625 lactose-fermenting cultures from normal stools, both animal and human.

hand and the feces themselves on the other, depends largely upon the fact that the nutrient conditions are ordinarily radically altered by the transfer to the fermentation media. This alteration in medium makes it possible for types of bacteria not obviously dominant in the feces, or, indeed, clearly in the minority, to gain a relatively prominent position under these conditions.

The fact that such a readjustment of types is liable to occur has important advantages and equally significant drawbacks. Without recognizing the disproportionate growth one might erroneously assume that a much larger portion of a certain flora is present than is the case; for example fecal fields may contain small numbers of *B. bifidus*, yet in the fermentation tubes they may be prominent. Again, the gas bacilli may undergo extensive multiplication in the fermentation tube despite the fact that the fecal material from which they were obtained contains them in moderate numbers only. Here again one sees the necessity for controlling the appearances obtained from the sediments of the fermentation tubes by means of cultures from the stools as well as by close examination of the Gram-stained fecal fields. Similar overgrowths occur with the coccal forms, *Mic. ovalis* (*enterocoque*), streptococci and staphylococci.

The disproportionate growth has its advantages as well as its disadvantages. Certain types which are significant although originally occurring in small numbers are thus brought to notice when otherwise they would be overlooked.

Experience has shown that overgrowths occurring during the first eighteen to twenty hours of incubation are due to the presence of significant numbers in the stools, capable of asserting themselves in the higher levels of the digestive tract, and capable of enormous proliferation under suitable nutrient conditions. An excellent example is again furnished by *Bact. Welchii.* In patients who have an infection of the intestinal tract with this organism there may be times of improvement when the numbers of this particular type in the feces is small, as shown by the microscopic examination of the fecal fields—so small, in fact, that if the observation were confined to the patient at this time, no suspicion would be excited of the existing tendency of overgrowth of these organisms in the intestine. Yet upon inoculation of the feces into fermentation tubes a prominent, active

growth of these organisms is very liable to occur under these conditions.

In contrast with this is the following observation. The fecal fields from normal nurslings and bottle-fed children commonly show a few organisms having the morphology of the gas bacillus. That these bacteria belong in the class of the gas bacilli is made probable through the fact that by inoculating relatively large amounts of the feces into rabbits' ear-veins, with subsequent incubation (Welch-Nuttall test) the typical gas-liver will be developed. Inoculations into fermentation tubes made from feces of this type of case have, in our experience, failed uniformly to show overgrowths of this bacillus.

It should be clearly understood that the presence of moderate or even considerable numbers of *Bact. Welchii* does not necessarily lead to overgrowth in the fermentation tube.

PRESS OF WILLIAMS & WILKINS COMPANY
BALTIMORE

NOTE ON THE PRODUCTS OF BACILLUS INFANTILIS GROWN IN ARTIFICIAL MEDIA

BY

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(Fellow of the Rockefeller Institute)

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BY

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Bacteriological studies of the feces in certain cases of arrested development in infancy[1] have shown this disorder to be associated with the presence of large numbers of the microörganisms to which we have given the name *B. infantilis*. Although the relation of this microörganism to the derangement of intestinal function is not yet clear it is evident that it is so prominent among the intestinal bacteria in this disease, even in its early stage, as to deserve careful study from every standpoint. The morphological and cultural characters of *B. infantilis* have been studied by one of us (Kendall) and we now desire to record here some observations which have been made on the bio-chemical properties of the bacillus.

The most noteworthy fact relating to the biological chemistry of *B. infantilis* is its ability to form volatile alkali. When grown for two weeks in plain broth at the body temperature this organism was found to have given rise to considerable quantities of volatile bases, consisting chiefly of ammonia. The quantity of volatile base formed in three different experiments carried on under approximately the same conditions was equivalent to 3.8 per cent, 4.2 per cent and 4.1 per cent normal sodium hydroxide, using alizarin as an indicator. It is interesting to observe that the formation of volatile alkali by *B. infantilis* was from three to four times greater than the amount made in plain broth by *B. coli* growing under essentially the same conditions for the same length of time. While the greater part

[1] See *Infantilism from Chronic Intestinal Infection*, by C. A. Herter, The Macmillan Company, 1908.

of the volatile bases formed by *B. infantilis* is neutralized by acids simultaneously formed (lactic and succinic, together with volatile acids, probably for the most part propionic and butyric) the bases after a few days predominate sufficiently to impart a decided alkaline reaction to the broth culture.

We are certainly justified in classing *B. infantilis* as one of the very active producers of ammonia. The differences just mentioned respecting the ability of *B. coli* and *B. infantilis* to make volatile bases are not attributable to differences in the growth of the bacteria in the two sets of culture. For while it is not possible to gauge [illegible]y the inequalities in rapidity of growth, a compari[illegible]urbidities, as well as of the microscopical appeara[illegible]d that we were dealing with fairly comparable rates [illegible]ction, as shown by the conditions at the end o[illegible]n period of two weeks.

The volatile alkali o[illegible]n broth cultures of *B. infantilis* does not consist e[illegible]mmonia. The use of Hoffmann's carbylamine [illegible]ved clearly that a primary amine is formed early i[illegible]rse of the decomposition. The development of an unm[illegible]rbylamine reaction has been a feature of all our alk[illegible]illates obtained from broth cultures of *B. infantilis*. [illegible] disposed to attribute this reaction to the presence of an alkylamine but it cannot be denied that the presence of diamines such as putrescine and cadaverine is not impossible in our distillates. Methylamine and ethylamine probably do not occur separately among decomposition mixtures and it is not unlikely that both are present in our distillates, the former perhaps preponderating. We have made no observations with a view to determining the amount of primary amines formed by *B. infantilis*.

In order to determine whether *B. infantilis* causes the putrefactive decomposition of proteids it was grown in broth for a period of three weeks or longer. The cultivation was carried on in flasks containing one liter of the culture medium and under such conditions as were likely to secure both aërobic and anaërobic development. No attempt was made, however, to secure such strict anaërobic conditions as are obtainable under hydrogen or under carbon dioxide. As there is a strong tendency for the organisms to collect on the surface of the culture medium

there may be some difficulty in securing good growth in the lowest part of the flask where the conditions are relatively anaerobic, but this difficulty was in a degree overcome by frequently shaking the receptacles. Under these experimental conditions we were unable to detect the presence, of indol, skatol, phenol, aromatic oxyacids, hydrogen sulphide or mercaptans. From acidified concentrated broth cultures and from milk cultures it was possible to obtain ethereal extracts containing material which gave the color reactions for indolacetic acid, but this derivative of tryptophan was not thus obtainable in amounts sufficient for identification. Assuming that we are justified from the color reactions in considering that indolacetic acid was formed, it is certain that it was present in only very slight concentration.

The addition of tryptophan to the broth medium did not yield indol or skatol from the action of *B. infantilis* nor did this addition lead to the formation of an increased amount of indolacetic acid. Similarly, the addition of tyrosin to the broth did not lead to the development of phenolic derivatives of tyrosin. Finally, the addition of cystin to the broth was not followed by the liberation of hydrogen sulphide or methyl mercaptan. From these experiments we have reached the conclusion that our organism does not possess putrefactive activities, at least under ordinary conditions of growth. But it is proper to say that different results may conceivably be obtained under strict anaërobic conditions. It is also possible that under states of symbiotic action with other bacteria *B. infantilis* may develop powers different from those which we have described. It is apparently a characteristic of infantilism from intestinal infection that the urine gives very strong reactions for aromatic oxyacids and we do not consider it impossible that our microorganism has a part in their formation, although the indications are at present opposed to this view.

When boiled with caustic potash or caustic soda the broth cultures of *B. infantilis* yield a strong reddish-brown color which corresponds to the characters of the Voges-Proskauer reaction. This reaction is only obtainable from cultures grown on media containing peptones or albumoses.

On media containing dextrose *B. infantilis* forms lactic acid,

succinic acid and volatile fatty acids. We have not detected the presence of alcohols, ketones or aldehydes.

It is a question of some interest whether so strong an alkali producer as *B. infantilis* may, by virtue of its production of ammonia, give rise to significant irritant action on the intestinal mucous membrane in those cases where the organism is in process of becoming parasitic and is present in very large numbers.

Finally it should be observed that the ether extract of old cultures of *B. infantilis* in broth, yields an abundance of an apparently fatty substance which we deem worthy of further study.

PRESS OF WILLIAMS & WILKINS COMPANY
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THE INFLUENCE OF DIETARY ALTERNATIONS ON THE TYPES OF INTESTINAL FLORA

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THE INFLUENCE OF DIETARY ALTERNATIONS ON THE TYPES OF INTESTINAL FLORA.

(Plates I–III.)

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The first systematic investigation of the fecal flora was that made by Escherich[1]. This observer, using modern aërobic and anaërobic cultural methods, isolated from the dejecta of normal nurslings and bottle-fed babies some of the best known of the intestinal bacteria. Subsequent workers have enriched the literature of intestinal bacteriology with scores of more or less imperfectly described and incompletely identified organisms, without, however, studying the newly-isolated bacteria either with respect to their numerical relations to other fecal bacteria or from the standpoint of their chemical activities within the intestine.

The discovery of the etiological relationships of the typhoid, dysentery and cholera organisms to these diseases also diverted attention from the normal intestinal bacteria, and at once directed the efforts of investigators toward the isolation of bacterial "species" which might be the causative agents of a variety of intestinal ailments.

In a previous communication[2] attention was directed to the chemical inactivity, or, more correctly, the inability of many exogenous pathogenic bacteria to produce deep-seated changes in artificial media. It would appear, inasmuch as the more promi-

[1] *Die Darmbakterien des Säuglings*, Stuttgart, 1886.

[2] Kendall, A. I.: This *Journal*, vi, p. 499, 1909.

nent normal bacteria are able to initiate marked changes in their environment, that there is a fundamental difference between these two types of bacteria. It is thus not difficult to see, that the lack of appreciation of this important difference is an element in causing the present unsatisfactory state of our knowledge of the intestinal bacteria.

The present studies are based upon an entirely different intent from that of previous investigators. Instead of isolating a series of cultures from the dejecta of man and animals, and studying them in the greatest detail, an attempt has been made to demonstrate definite relations between the nature of the diet on the one hand, and the leading characteristics of the resulting bacteria on the other, including some products of their vital activity.

There are many clinical indications in man that sharp alternations in the chemical nature of the diet are attended by rapid changes in the physiological state of the digestive tract. For example it is well known to physicians that the free use of carbohydrates (e. g., lactose) in bottle-fed infants is commonly followed by a softening in the consistence of the feces and perhaps by the signs of excessive fermentation. The return to a milk diet restricted in carbohydrates is followed by a prompt recession of these signs of increased fermentation. Similarly it is a familiar observation that certain undesirable symptoms (lassitude, drowsiness, headache) attendant on the use of an excessive share of protein in the diet are relieved by a restriction of proteins and a freer use of carbohydrates. Although it has sometimes been assumed that alterations in the bacterial flora attend such changes in diet, we know of no studies designed to determine the precise effects on the nature of the flora that may arise from definite and abrupt variations in the chemical composition of food. In our experiments we have asked ourselves if it is possible to establish quite definite unchallengeable concomitant variations in food and bacteria, since the establishment of such variations, under physiological conditions, must be an essential condition for the discovery of exact indications for diet under states of infection in the intestine. In order to secure conditions as favorable as possible to clarity and definition of results we have planned our experiments so as to obtain somewhat extreme

physiological dietaries. Thus we have alternated a diet consisting mainly of protein with a diet in which carbohydrates have been more than ordinarily prominent and vice versa. We have furthermore made the alternations abrupt in order to leave the least possible chance for gradual adaptation to a given class of dietary.

These experiments were carried out upon two widely different types of mammals—monkeys and cats. The difference in the types of experimental animals selected served to test the question whether the same alternations in diet would yield the same results in bacterial change in omnivora and carnivora. The advantage of using monkeys as experimental animals, similar to man in their digestive processes, is obvious.

The diets selected consisted of meat for the cats and eggs for the monkeys as representative of protein foods. Milk to which was added dextrose was selected as the carbohydrate diet, experience having shown that this milk and sugar combination exhibits, from a bacterial point of view, the properties of carbohydrate food.

At first sight it would appear that eggs are an unusual diet for monkeys and that the results might be different from those which would obtain in normal primates, but it should be remembered that bird's eggs are by no means an uncommon delicacy in the diet of normal monkeys. Milk forms an essential part of the pabulum of these animals in their early life. Both meat and milk are common articles of food in the dietary of cats and need no further comment. As will be shown later, the striking fact brought out by these experiments is that the same bacterial changes occur with the same types of diet in the different types of animals irrespective of the apparent novelty of the food. And because of this observed regularity of relation between the nature of the diet and the character of the intestinal flora the results are all the more noteworthy.

The methods used in this investigation, described in a previous communication[1] differ from those previously described, not in their nature, but in the manner of utilizing them. Cultural media of appropriate composition have been used as specific

[1] Kendall, A. I.: *loc. cit.*

enrichment media in such a manner as to indicate the relative distribution of the acidophilic and protein flora as the diet is changed from carbohydrate to protein and vice versa.

The use of fermentation tubes containing dextrose, lactose and saccharose broth, as well as milk fermentation tubes has been particularly helpful, and the study of the Gram-stained sediments from these tubes, particularly the broth tubes, has furnished clues which have led, through the mediation of plating, to the emergence of valuable data.

It should be stated that the fecal flora are less responsive, or, better, respond less c[illegible] the earlier changes in diet, although even in the [illegible]s in diet the variations are strongly marked. As [illegible]ents are continued, there is a tendency toward a u[illegible] the flora, due apparently to the gradual suppressio[illegible]ganisms which are less easily habituated to the prot[illegible]ohydrate regimen. While at first sight this might s[illegible] source of error, further consideration shows that t[illegible] is in itself strong presumptive evidence of the corr[illegible]e results obtained. As the diet is changed from the [illegible] the carbohydrate, and back again the flora exhibit [illegible]r and stronger tendency to become simplified, until [illegible]e protein bacteria and the acidophilic bacteria crowd out the more or less ubiquitous organisms and the flora eventually respond with precision and promptitude to the changed dietary conditions.

The general plan followed was to place the animals upon a protein diet for one or two weeks, then to shift abruptly to the carbohydrate regimen for the same length of time, reversing the diets at regular intervals as indicated above. These intervals were found to be sufficiently separated to permit of the full development of the respective flora. At the same time they were found to be sufficiently far apart to prevent confusion of the two types of bacterial response to the dietary changes, such as is liable to occur when the alternations of diet are made too frequently. In the latter event the bacterial response reflects in part the protein regimen, in part the carbohydrate, and the results cease to be clear cut.

PHYSIOLOGICAL EFFECTS OF DIET.

As the proteolytic bacteria become dominant in the alimentary canal, the monkey becomes sleepy and rests on its perch with its head bowed in its hands; it is stupid and responds slowly to external stimuli, takes its food very deliberately, and manifests little interest in its surroundings. Not infrequently the animal even after a hearty meal will spend much time trying to bite the woodwork of its cage.

The urine is voided in small amounts daily and is relatively highly colored. The amount may, roughly, be considered to be one-half that which is obtained from a carbohydrate diet such as employed. In the urine as the proteolytic bacteria become fully established there is an abundance of indican as well as aromatic oxyacids. The urorosein reaction due to indolacetic acid is rarely observed and at best very faintly. The lower specific gravity of the urine during the milk periods has to be taken into account in estimating the intensity of the reactions for indican and aromatic oxyacids. No observed differences in the concentration of the urines of the different periods can account for the variations in these reactions which were actually observed.

The fecal mass is small, rather desiccated and distinctly yellow in color or yellowish-brown. The odor is strongly suggestive of indol or skatol.

As the diet is changed to carbohydrate (best done by feeding the animal milk containing a moderate amount of dextrose), both the psychical and the physical attitude of the animal undergo a great change. The monkey no longer holds its head in its hands, the posture is erect, the animal is alert and bright, notices everything, reacts promptly to all kinds of stimuli and eats abundantly. The eyes lose their dull, lustreless appearance and become bright. The animal no longer attempts to chew the woodwork of its cage.

The urine becomes more voluminous, approximately twice its previous volume and pale in color. The indican and aromatic oxyacids grow much less or disappear. Any traces of indolacetic acid also disappear.

The feces, at first diarrhœal become formed, gray in color, and

inoffensive in odor, contrasting markedly in this respect with the feces derived from a protein diet.

BACTERIOLOGICAL CONDITIONS.

Morphology. As the "protein flora" becomes established the Gram-stained fields show considerable numbers of large, Gram-positive bacilli with rounded ends, which rarely or never occur in chains but not infrequently occur in pairs. Smaller Gram-positive organisms also with rounded ends, but showing a distinct tendency to b[illegible]shaped particularly as sporulation approac[illegible] The latter, and to some extent the former, ma[illegible]itions, particularly when there is a change [illegible] negative and show undoubted signs of dege[illegible]eration is usually accompanied by vacuolation. [illegible]am-positive organisms, Gram-negative organisms[illegible]rphologically, to *B. coli*, are abundant. O[illegible]n time to time make their appearance; the[illegible]organisms, however, are inconstant, and appare[illegible]arded as of secondary importance.

Cultural Features. [illegible] fecal flora, when inoculated into dextrose, lactose and saccharose fermentation media, produce typically considerable volumes of gas, not infrequently amounting to 90 or even 100 per cent of the length of the closed arm of the fermentation tube. The fermentation medium becomes very turbid, and an abundant sediment collects at the foot of the closed arm. This sediment, stained by Gram's method, shows the same types of organisms that were present in the Gram-stained smears prepared from the feces direct. Fermentation tubes containing milk also show an abundance of gas. The milk is very considerably peptonized and usually the undissolved coagulum is colored brown. As a rule no distinct odor of butyric acid is detectable in either the carbohydrate fermentation tubes or the milk fermentation tubes. Gelatin stab cultures inoculated as before with the mixed fecal flora, show somewhat rapid peptonization. This peptonization usually assumes the form of a rather deep funnel, indicating that the bacteria bringing about the change are not obligate anaërobes. The acid dextrose broth shows few bacteria of the acidophilic type.

The transitional flora, as the animal changes from protein to carbohydrate is characterized by two noteworthy features; first the bacteria become much smaller and stain rather poorly. Evidences of degeneration are seen, particularly vacuolation, in certain instances spore-formation has been a feature as well. Secondly, great irregularities in the distribution of the various types occur. These irregularities, which are not only morphological but are found to be cultural as well, are apparently due to the antagonism which occurs between the protein and the carbohydrate flora. As the latter gradually becomes dominant, the Gram-stained fields which, on the protein diet were heterogeneous in appearance, tend to become more homogeneous and the most prominent organisms are Gram-positive rods, thinner than those noted in the protein diet, and somewhat longer. These rods, indeed, are so abundant that the fields resemble in a striking manner those of normal nurslings. Cultural investigation has shown that these organisms are in reality closely allied to those characteristic of the normal nursling flora. It is only by careful scrutiny that a few residual organisms of the protein diet, particularly those resembling the smaller, Gram-positive organisms described above, can be found.

Culturally the conditions are exactly the reverse of those obtaining on the protein diet. In the fermentation tubes the gas volume rapidly decreases and ultimately practically disappears. The turbidity becomes very much less marked and, indeed, in some cases only a faint opalescence develops. The sediments are composed almost wholly of rather elongated, Gram-positive rods, agreeing with those found in the feces. In addition, under certain not well understood conditions, rod-shaped organisms with bifid ends are seen. These organisms in reality are to be regarded as *B. bifidus*. In several instances it has been possible to isolate these bifid-like organisms and in their cultural complex and general morphology they are to be regarded as identical with Tissier's organism.

The milk fermentation tubes show a very slight growth, and in fact, the only visible indication of bacterial activity is a slight coagulation. The organisms of the carbohydrate flora do not cause pronounced peptonization or free gas-formation in this medium. In gelatin the growth is very scanty and only after

several days is it usual to find signs of bacterial development. The organisms which occur are to be regarded as proteolytic bacteria which have developed in this medium.

Acidophilic bacteria do not ordinarily grow in gelatin and the value of the gelatin tubes in this connection is principally that they furnish an environment so favorable to the proteolytic flora and so unfavorable to the carbohydrate flora that the former, even when present in very small numbers, and with greatly diminished vitality, can grow. In the acid dextrose broth the acidophilic bacteria find a very favorable medium and even in the highest acidities they grow readily, contrasting in this respect with the lack of growth in the medium which is so characteristic of the protein flora.

Much attention was given to the question of the presence of strict anaërobes, especially the gas bacillus (*B. perfringens*, *B. aërogenes capsulatus*) as it is now well known that organisms of this type are liable to be present in the intestine of many animals. Many attempts were made to isolate the gas bacillus from the feces of the experimental monkeys but without success. In one instance (Monkey No. III) the animal was killed after having been fed on a protein diet and cultures were made from various levels of the intestinal tract. Material obtained from various levels in the large and small intestine was suspended in sterile salt solution and injected into the ear veins of rabbits. The animals were killed after a short interval and incubated at 30° for eighteen hours. The livers were found to be dark colored and to have a putrefactive odor but not the odor of butyric acid. There was no development of gas in the livers. The microscopical appearances obtained in smears made from the liver were like those obtained from the liver of an animal subjected to the Welch-Nuttall procedure after the intravenous injection of a culture of the gas bacillus. The bacteria were very abundant and had the morphological appearance of the gas bacilli. The appearances indicated that they were present in almost pure culture. Subsequent cultural study, however, made it clear that these organisms could not be classed with the gas bacilli. Inoculated into milk fermentation tubes they failed to induce the characteristic stormy fermentation with butyric acid production which one expects to find where one is dealing with the gas

bacillus. Moreover our organisms grew aërobically on dextrose-agar plates. They liquefied gelatin. We are strongly disposed to class these organisms observed by us as members of the *B. subtilis* group.

Pure cultures of the organisms which we have just described were injected into the leg muscles of guinea-pigs. They gave rise to a moderate hemorrhagic œdema at the site of inoculation. The bacilli were recovered in pure culture from the site of lesion and again inoculated into rabbits by the Welch-Nuttall method. It was again found that they had multiplied very abundantly under anaërobic conditions in the liver and presented an appearance like that observed where the gas bacillus is present. Again they failed to induce gas production in the liver.

While these experiments do not absolutely exclude the presence of the gas bacillus in the intestinal contents of our monkeys, they render it highly probable that this organism was not an important feature in the intestinal contents of the monkeys fed on a protein diet. In the case of the kittens no careful study was made to determine the presence of the gas bacillus. It is not unlikely from what we know of the intestinal bacteria in cats on a meat diet that this organism was present. Our experience in the case of the monkeys showed us, however, that the presence of the gas bacillus is not essential to an explanation of the phenomena which we have described in this study.

The tables are for the most part self-explanatory. The gas produced in the dextrose, lactose and saccharose tubes is expressed in percentages of the total length of the tube, instead of millimeters, experience having shown that the former method is more accurate. The readings were made at the end of eighteen hours incubation at 37° .

The term "type" occurs in two different places; the first in connection with the fermentation tube sediments, and directly stained smears, in which the +, – and ± mean, respectively, positive staining organisms (gram stain), negative staining organisms, and (in the ± fields) an approximately equal division between the positive and negative types. In other words, the + and – indicate the dominance of Gram-positive and Gram-negative bacteria respectively. In the fermentation tubes, associated with *gas*, the term is used to indicate the amount of

In Table V (Monkey II) is presented an experiment in which the diet was changed from bread and bananas to milk, then to eggs, then back to milk, then to eggs and back again to milk. It is not necessary to comment in detail on this table. It shows essentially the same variations as those observed in the kittens when the diet was shifted from one of milk to one of eggs; or from eggs to milk. The same phenomena as were observed in the case of the kittens, when the change was made from meat to milk or vice versa, are observed here, namely, variations of the acidophiles, in the organisms of the subtiloid type, and variations in gas production. Very striking is the abrupt rise in the gas production under the influence of eggs and its subsequent fall under the influence of milk and sugar. The records as regards the putrefactive substances in the urine are incomplete but point to a decline in such products during the milk period.

Table VI (Monkey III) like the preceding one, records the results of successive alternations in diet from milk to eggs. Essentially the same comments as apply to Table V (Monkey II) find place here. Again the effect of the diet on the acidophiles and subtiloid organisms and the gas production is very marked. Although there are some irregularities in the gas production, the figures of the table indicate in a convincing way the influence of the protein diet in causing a rise in the formation of gas and the contrary influence of the milk and sugar diet. A similar influence is seen in the rise of gas production on a milk medium under the influence of the egg diet.

Table VII (Monkey IV) records another experiment illustrating the effect of change from a milk to an egg diet. In this experiment the acidophilic bacteria did not appear in connection with the milk diet, and it is also noteworthy that the subtiloid organisms do not appear to have been as much affected by the changes in diet as in previous experiments, although the fermentation tube sediments showed an increase of the large subtiloid forms during the egg diet. The organisms of the *B. coli* group also appear to have been less affected than usual by the dietary changes. On the other hand the gas production in the fermentation tubes shows in a striking way the typical effects of the change from milk to egg diet. The gas production was very low throughout the milk diet and promptly rose to a high level and maintained this high level throughout the egg period. Toward the end of the egg period there was a rise in the indican. The influence of the bacteria from the milk period and the egg period respectively on milk medium was less in this experiment than in the others that have been recorded.

One of the most notable features of our investigation is the contrast in the gas production correlated with the changes in diet. We deem it desirable to say that although we have given this phenomenon considerable attention we are still without an explanation which is satisfactory. At first it seemed possible

that the high gas values for protein diet were due to the activity of the gas bacillus (*B. aërogenes capsulatus*) but, as already stated, we were unable to prove the presence of this organism in the intestine of monkeys on protein diet. Experimental combinations of the prominent subtiloid organisms, already mentioned, with colon bacilli failed to give gas volumes approximating those which are recorded in our table. The possibility of the presence of the abundant gas formers, *B. cloacæ*, has not been fully excluded.

In addition to the studies on kittens and monkeys some observations were made by one of us (H) on a human subject in good health, with a view to determining the influence of the addition of from 100 to to 200 grams of cane sugar daily to the ordinary mixed diet containing meat. Under this addition of sugar the feces became soft and acid in reaction and odor, and the indol, skatol and phenol were diminished markedly. The acidophilic types of bacteria were distinctly increased. The influence (if any) exerted on other kinds of bacteria was not studied. The gas production by the mixed fecal flora, grown on saccharose, lactose and dextrose bouillon was markedly diminished, but the depression was from a lower level than in the case of the experiments on kittens and monkeys.

One of us (H) has made the following observations on the influence of alterations in diet on the composition of the intestinal contents with regard to putrefactive products, especially indol, skatol and hydrogen sulphide.

In a monkey receiving two eggs daily there was a rise in the amount of indol and in the amount of skatol detectable. The reactions after reaching a maximum several days after the use of this diet, continued strong during the remainder of the ten day period on this diet. On changing the diet to one consisting of 750 cc. of milk and 10 grams of dextrose daily, there was a prompt fall in the intensity of the indol and skatol reactions obtainable from the intestinal contents. After four days the reactions of these substances were only slight; after seven days, only the faintest traces were observed under conditions comparable to the preceding test.

The following values were obtained for the hydrogen sulphide bound to the feces under varying conditions of diet:

TABLE I.

Kitten A.

DATE.	FECES. GRAM STAIN.											FERMENTATION TUBE SED GRAM STAIN.						
	Bifidus.	Acidophilus.	Large subtiloid.	Small subtiloid.	Spores.	Coli.	Cocci.	Long chains.	Short chains.	Spiral forms.	Type.	Bifidus.	Acidophilus.	Large subtiloid.	Small subtiloid.	Spores.	Coli.	Cocci.
VII—13	−	+	|	|	−	?	|	−	−	+	+	++	+	|	|	|	−	−
14	−	+	|	|	−	−	|	−	−	+	+	+	+	|	|	−	−	−
17	−	+	−	|	−	−	|	−	−	+	+	+	+	|	|	−	−	−
18	−	+	|	|	−	−	|	−	−	+	+	+	+	|	|	−	−	−
19	−	+	|	|	−	−	|	−	−	+	+	|	++	|	|	−	−	−
21	|	+	|	|	−	|	|	−	−	+	+	−	+	+	+	−	+	+
24	−	|	+	+	−	+	|	−	−	+	±	−	|	+	+	−	+	|
26	−	−	++	+	+	+	+	−	−	+	±	−	|	++	+	+	+	|
30	−	−	++	+	|	++	+	−	−	+	±	−	|	++	+	+	+	|
VIII—2	−	−	++	+	+	++	+	−	−	+	±	−	?	++	+	+	++	|
3	−	−	++	+	+	++	+	−	−	+	±	−	?	++	+	|	++	−
5	−	−	++	+	−	++	|	−	−	+	±	−	−	++	+	|	++	|
7	−	−	++	+	+	++	|	−	−	+	±	−	−	++	+	|	++	|
9	|	−	++	+	−	++	+	−	−	+	±	−	−	++	+	|	++	+

TABLE I—Continued

…TION	URINE.			MILK.			DIET.	REMARKS.
Type of growth.	Indican.	Indolacetic acid.	Millon's.	Coagulated.	Gas.	Peptonized.		
+							Milk and dextrose.	
+							"	
+	−	−	−				"	
+							"	
+	−	−	−				"	Meat begun.
+							Meat	VII—22
++	++	−	+++				"	
++	++	−	+++	+++	100	+	"	
++	+	−	+++	+++	100	+	"	
++			−				"	
++							"	
++	+	−	+++				"	
++							"	
++							"	

TABLE II.

Kitten B.

Date.	Feces. Gram stain. Bifidus.	Acidophilus.	Large subtiloid.	Small subtiloid.	Spores.	Coli.	Cocci.	Long chains.	Short chains.	Spiral forms.	Type.	Fermentation tube sedimen[t] Gram stain. Bifidus.	Acidophilus.	Large subtiloid.	Small subtiloid.	Spores.	Coli.	Cocci.	Long chains.	Sh
VII—18..	—	—	+	+	—	+	—	—	—	—	±	—	?	+	—	—	+	+	—	—
22..	—	—	++	+	++	+	—	—	—	—	±	—	?	++	+	+	+	—	—	—
23..	—	?	++	+	—	+	+	—	—	—	±	—	—	++	+	—	++	—	+	—
25..	?	—	++	+	+	+	—	—	—	+	±	—	—	+++	+	—	+	—	+	—
26	—	—	++	+	+	+	—	—	—	+	±	—	—	+++	+	—	++	—	—	—
27..	—	—	++	+	+	+	—	—	—	+	±	—	—	+++	+	—	+	—	—	—
29..	—	—	+++	+	+	+	—	—	—	+	±	—	—	+++	++	—	++	+	—	—
30..	—	—	++	+	—	++	—	—	—	+	±	—	—	++	++	—	++	+	—	—
VIII— 4.	—	—	++	+	—	++	—	—	—	+	±	—	—	+++	++	—	++	++	—	—
6.	—	—	++	+	—	+	—	—	—	+	±	—	—	+++	++	—	++	++	—	—
9.	—	—	++	+	—	+	—	—	—	+	±	—	—	+++	++	—	++	++	—	—
12	—	—	++	+	—	+	—	+	—	+	±	—	—	+++	++	—	++	++	—	—
13.	—	—	++	+	—	++	—	—	—	—	±	—	—	++	++	—	++	++	+	—
14	—	+	++	+	—	++	—	—	—	—	±	—	—	++	++	+	++	—	—	—
16.	—	+	++	+	—	++	+	+	—	++	±	—	—	++	++	—	++		+	—
18..	—	+	++	+	—	++	—	—	—	+	±	—	++	+	+	—	+	—	—	—
20..	—	++	—		—	—	—	—	—	++	+	—	++	—	—	—	—	—	+	—
23..	—	++	—	—	—	?	—	—	—	+	+	++	++	+	—	—	?	—		—
24—	—	++	+	—	—	+	—	—	—	+	+	—	++	+	—	—	—	—	—	—
27..	—	+	+	—	—	—	—	—	—	+	+	—	++	+	—	—	—	—	—	—
30	—	+	++	+	—	—	—	—	—	+	+	—	++	+	—	—	+	—	—	—

TABLE II—Continued.

Type of growth.	URINE. Indican.	Indolacetic acid.	Millon's.	MILK. Coagulated.	Gas.	Peptonisation.	DIET.	REMARKS.
±	+	–	+++				Meat	Feces dark brown tarry
-+	++	–	++	++	80	+	"	"
+	|	–	+++				"	"
-+	–	–	+++				"	"
-+	++	–	+++				"	"
-+							"	"
-+	+++	–	+++	++	100	+	"	"
-+							"	"
-+	++		+++	++	100	+	"	"
-+							"	"
-+								"
-+	++	–	+++	++	90	+	Milk	Feces light brown, some mucus—soft
-+	|	–	+++					Feces light brown—watery
-+	|	–	+++				"	Feces light brown—soft
-+							"	"
+	–	–	–	+	60	+	"	"
+	–	–	–	–			"	"
+							"	Feces gray brown-soft
+	|	–	–	+	5		"	"
+							"	"
+	–	–	–	+	–	+	"	"

TABLE III.

Kitten C.

DATE.	FECES. GRAM STAIN.											FERMENTATION TUBE SEDIM GRAM STAIN.							
	Bifidus.	Acidophilus.	Large subtiloid.	Small subtiloid.	Spores.	Coli.	Cocci.	Long chains.	Short chains.	Spiral forms.	Type.	Bifidus.	Acidiphilus.	Large subtiloid.	Small subtiloid.	Spores.	Coli.	Cocci.	Long chains.
VII—17.......	−	|	|	|	−	+	+	−	−	|	±	−	?	+	+	−	+	|	−
VII—18.......	−	|	|	|	−	+	+	−	−	|	±	−	?	+	+	−	+	+	−
VII—20.......	−	?	+	+	−	+	+	+	−	|	±	−	−	++	+	−	++	|	+
VII—23.......	−	−	++	+	+	+	−	−	−	+	±	−	−	++	+	−	++	|	+
VII—25.......	−	−	++	+	|	+	+	−	−	−	±	−	−	+++	+	−	++	|	+
VII—26.......	−	−	++	+	+	+	±	−	−	+	±	−	−	+++	+	−	++	+	−
VII—27.......	−	−	++	+	|	+	−	−	|	+	±	−	−	+++	+	−	++	+	−
VII—30.......	−	−	++	−	−	+	+	−	|	+	±	−	−	+++	+	−	++	+	−
VII—31.......	−	−	++	+	+	++	+	−	+	+	±	−	−	+++	+	−	++	++	−
VIII— 1.......	−	−	++	+	−	++	+	−	−	+	±	−	−	+++	+	−	++	+	−
VIII— 2.......	−	−	++	+	−	++	+	−	−	+	±	−	−	+++	+	−	++	+	
VIII— 4.......	−	−	++	+	−	+	|	−	−	+	±	−	−	+++	+	−	++	+	
VIII— 6.......	−	−	++	+	|	++	|	−	−	+	±	−	−	+++	++	−	++	+	
VIII— 9.......	−	−	++	+	+	++	|	−	−	+	±	−	−	+++	++	+	++	++	
VIII—11.......	−	−	++	+	|	++	|	−	−	+	±	−	−	+++	++	|	++	++	
VIII—14.......	−	−	++	+	+	++	+	−	−	+	±	−	−	+++	++	−	++	+	
VIII—18.......	|	−	++	+	−	++	|	−	−	|	±	±	+	++	|	−	+	+	
VIII—20.......	?	++	|	|	−	|	−	−	−	++	+	−	+	++	|	+	+	−	
VIII—23.......	−	++	|	|	−	|	−	−	−	++	+	|	+	+	|	−	+	−	
VIII—24.......	−	+	|	|	−	|	−	−	−	+	+	−	+	+	|	−	|	−	
VIII—26.......	−	++	−	−	−	−	−	−	−	+	+	+	+	|	−	−	|	−	
VIII—28.......	−	++	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	
VIII—30.......	−	++	−	−	−	−	−	−	−	+	+								

TABLE III—Continued

ENTATION UBES.		URINE.			MILK.			ACIDOPHILES, ACETIC ACID BROTH.			DIET.	REMARKS.
Saccharose.	Type of growth.	Indican.	Indolacetic Acid.	Millon's.	Coagulated.	Gas.	Reaction.	5 per cent.	10 per cent	20 per cent.		
90	++	++	−	+++	+	*p. c.*		++	++	+	Meat	
65	++				+++	95	+				"	
95	++	+	|	+++				++	+	−	"	
40	++	+	−	+++							"	
60	++	+	−	+++							"	
70	++	+	−	+++	++	85	++	++	+	−	"	
80	++	+++	−	+++	++	100	++				"	
45	++	++	−	++	++	100	++	+	−	−	"	
65	++	+	−	++	++	100	++				"	
45	++	+	−	++							"	
90	++	+	−	++							"	
100	++	+	−	+							"	
85	++	+	−	+++							"	
65	++	++	−	+++							"	
80	++										"	
85	++	++	−	+++							Milk and dextrose	
20	++	−	−	−	++	20	+					
15	++	−	−	−							"	
10	++	−	−	−							"	
30	++	−	−	−	+	5	++				"	
2	+	−	−	−							"	
1	+	−	−	−	+	−	++				"	
0	+										"	

TABLE IV.

Monkey 1 (Control Monkey)

FECES. GRAM STAIN.

Date.	Bifidus.	Acidophilus.	Large subtiloid.	Small subtiloid.	Spores	Coli.	Cocci.	Long chains.	Short chains.	Spiral forms.	Type.
VI— 8	−	−	+	+	+++	+	+	−	−	−	±
9	−	−	+	+	++	+	+	−	−	±	+
10	−	−	+	+	++	+	+	−	−	±	±
11	−	−	+	+	+	+	+	−	−	±	±
12	−	−	+	+	−	+	+	−	−	−	±
13	−	−	+	+	−	+	++	−	−	+	±
14	−	−	+	+	+	+	++	−	−	+	±
15	−	−	+	+	+	+	+	−	−	+	±
16	−	−	+	+	+	+	−	−	−	+	±
17	−	−	+	+	−	+	−	−	−	+	+
18	−	−	−	+	+	−	+	−	−	+	±
19	−	−	+	+	−	+	−	−	−	+	±
20	−	−	+	+	−	+	−	−	−	+	±
21	−	−	+	+	−	+	+	−	−	−	±
23	−	−	−	+	−	+	+	−	−	−	±
24	−	−	+	+	−	+	+	−	−	−	±
25	−	−	+	+	−	+	+	−	−	−	±
26	−	−	++	+	−	++	+	−	−	−	±
27	−	−	+	+	+	+	−	−	−	−	±
28	−	−	++	+	+	+	−	−	−	+	±
29	−	−	++	+	+	+	−	−	−	−	±
30	−	−	++	+	−	+	−	−	−	−	±
VII— 1	−	−	++	+	−	+	++	−	−	+	±
2	−	−	++	+	−	+	++	−	−	−	±
3	−	−	++	+	−	+	+	+	−	++	±
4	−	−	++	+	−	+	−	−	−	+	±
5	−	−	++	+	−	+	+	−	−	+	±
VII— 6	−	?	++	+	−	+	+	−	−	−	±
7	−	?	+	−	−	+	−	−	−	−	±
8	−	−	+	−	−	+	−	−	−	+	±
9	−	+	+	+	−	+	+	−	−	+	±
10	−	−	++	+	−	+	−	−	+	+	±

FERMENTATION TUBES SEDIMENTS. GRAM STAIN.

Date.	Bifidus.	Acidophilus.	Large subtiloid.	Small subtiloid.	Spores.	Coli.	Cocci.	Long chains.	Short chains.	Spiral forms.	Type.
VI— 8	−	−	++	+	−	+	+	−	+	−	±
9	−	−	−	−	−	+	+	−	−	−	±
10	−	−	−	−	−	+	+	+	+	++	±
11	−	−	++	++	−	−	+	+	+	+	±
12	−	−	++	+	+	+	+	−	−	±	±
13	−	−	++	+	−	+	+	−	−	+	±
14	−	−	++	+	−	−	+	−	−	+	+
15	−	−	++	+	−	+	+	−	−	+	±
16	−	−	++	−	−	+	−	+	−	++	±
17	−	−	++	−	−	+	−	+	+	+	±
18	−	?	+	+	−	+	+	−	+	++	±
19	−	−	+	+	−	+	−	−	−	++	±
20	−	−	+	+	−	+	−	−	−	++	+
21	−	−	+	+	−	+	−	+	−	+	±
23	−	−	+	+	−	+	+	+	+	+	±
24	−	−	+	+	−	++	+	+	−	±	±
25	−	−	+	+	−	++	+	+	−	±	+
26	−	−	+	−	−	+	++	+	+	±	
27	−	−	+	+	−	−	+	+	−	+	±
28	−	−	+	−	−	+	++	+	−	−	±
29	−	−	+	−	−	+	++	+	+	−	±
30	−	−	+	−	−	+	++	−	+	+	±
VII— 1	−	−	++	−	−	+	+	+	−	−	±
2	−	−	++	−	−	+	+	++	−	+	±
3	−	−	+	−	−	+	++	+	−	+	±
4	−	−	+	+	−	+	+	+	−	+	±
5	−	−	++	+	−	+	++	+	−	+	±
VII— 6	−	+	+	−	−	+	+	−	+	+	±
7	+	+	+	−	−	++	+	+	+	++	±
8	−	+	+	−	−	++	+	+	+	++	±
9	−	+	+	−	−	++	+	+	+	++	±
10	−	−	++	+	−	+	−	−	+	+	±

TABLE IV.—Continued.

ON	URINE.			MILK.			ACIDOPHILES; ACETIC ACID BROTH.			DIET.	REMARKS.
Type of growth.	Indican.	Indolacetic acid.	Millon's.	Coagulated.	Gas.	Peptonized.	5 per cent.	10 per cent.	20 per cent.		
++										Mixed diet; bread & bananas.	
++										" "	
++				+	80	+	+	+	|	" "	
++				+	90	+				" "	
++				+	60	+				" "	
++				+	5	|				" "	
++				+	10	−				" "	
++				+	5	−				" "	
++				+	5	−				" "	
++				+			++	−	−	" "	
++							+	−	−	" "	
++				+	5	−				" "	
++				+	5	−				" "	
++							+	−	−	" "	
++							+	−	−	" "	
++				+	20	−	|	−	−	" "	
++				−	−	−	|	−	−	" "	
+				+	10	−	|	−	−	" "	
++				+	15	−	|	−	−	" "	
++							|	|	−	" "	
++							|	+	+	" "	
++										" "	
+										" "	
+										" "	
++							+	+	+	" "	
++										" "	
++										" "	
++	−	?	?	+	20	+	+	+	+	" "	
++	−	−	−							" "	
++	−	−	|							" "	
++										" "	
++										" "	

TABLE V.

Monkey II.

DATE.	FECES. GRAM STAIN.											FERMENTATION TUBE SEDIMENTS. GRAM STAIN.										
	Bifidus.	Acidophilus.	Large subtiloid.	Small subtiloid.	Spores.	Coli.	Cocci.	Long chains.	Short chains.	Spiral forms.	Type.	Bifidus.	Acidophilus.	Large subtiloid.	Small subtiloid.	Spores.	Coli.	Cocci.	Long chains.	Short chains.	Spiral forms.	Type.
VI—7.....	−	+	|	+	−	|	+	+	|	|	±	−	|	|	|	−	|	+	−	−	−	+
8.....	−	+	|	+	+	|	+	+	|	|	±	−	+	|	|	−	|	+	+	+	+	+
9.....	−	+	+	+	−	+	+	−	−	+	±	−	+	+	|	−	|	|	|	|	+	+
10.....	−	−	+	+	+	+	+	−	−	+	±	−	−	+	|	−	+	|	+	+	+	+
11.....	−	−	+	+	|	+	+	−	+	+	±	−	−	+	|	−	+	+	+	+	++	+
12.....	−	−	+	+	|	+	+	|	|	|	±	−	−	+	|	−	+	+	−	+	+	+
13.....	−	−	+	+	|	+	+	|	|	|	±	−	−	+	|	−	|	++	−	−	+	+
14.....	−	−	++	+	|	+	++	|	−	−	±	−	−	+	+	−	+	++	−	+	+	+
15.....	−	−	++	|	|	+	++	|	−	−	+	−	−	+	+	−	+	++	−	|	+	+
16.....	−	+	++	+	|	+	+	−	|	+	±	−	−	+	+	−	+	|	−	−	+	+
17.....	−	+	++	+	+	+	+	−	|	+	±	−	−	+	+	−	+	+	+	+	+	±
18.....	−	|	+	+	−	+	+	−	+	+	±	−	+	|	+	−	|	+	|	+	+	±
19.....	−	|	+	+	|	|	+	−	+	+	±	−	+	+	+	−	|	+	|	+	+	+
20.....	−	−	+	+	|	+	+	|	+	+	±	−	−	+	+	−	+	+	|	+	+	+
22.....	−	|	?	+	−	−	|	−	|	++	+	++	−	+	|	+	−	|	+	−	+	+
23.....	−	|	?	+	−	|	−	−	|	++	+	−	+	?	+	−	|	+	−	|	+	+
24.....	−	|	?	+	−	|	−	−	|	++	+	−	+	|	+	−	+	+	−	|	+	+
25.....	−	|	|	+	−	|	−	−	|	++	+	−	+	−	|	−	−	++	−	|	+	+
26.....	−	+	|	+	−	|	−	−	|	++	+	−	+	−	|	−	−	|	−	−	++	+
27.....	−	+	|	|	−	?	−	−	|	++	+	−	+	−	|	−	|	+	−	−	|	+
28.....	−	+	|	|	−	|	−	−	|	+	+	+	+	−	|	−	|	|	|	−	++	+
29.....	−	+	|	|	−	|	−	−	|	+	+	++	+	−	−	−	|	|	+	−	++	+
30.....	−	+	|	+	−	|	−	−	|	+	+	|	+	|	−	|	−	|	−	−	++	+
VII—1.....	−	+	|	+	−	|	+	−	|	+	+	|	+	|	−	−	−	−	−	−	++	+
2.....	−	+	|	+	|	+	−	|	+	+	+	++	++	|	−	−	−	−	−	−	+	+
3.....	−	+	+	−	−	−	−	−	+	+	±	++	++	|	−	−	−	?	|	−	|	+
4.....	−	+	|	−	−	|	−	−	|	+	+	++	+	|	−	|	−	−	|	−	+	+
5.....	−	+	−	−	−	|	−	|	|	+	+	++	+	|	−	|	−	|	−	|	+	+
VII—6.....	−	+	|	|	|	+	+	−	|	|	+	++	+	|	|	−	+	|	−	−	+	+
7.....	−	+	|	|	−	|	|	−	−	+	+	++	+	|	|	−	|	−	−	−	+	+
8.....	−	+	−	−	−	|	−	−	−	+	+	−	+	|	|	−	+	|	−	−	++	+
9.....	−	+	+	|	−	+	|	−	|	+	+	+	+	−	−	−	+	|	−	−	++	+
10.....	−	|	+	|	−	+	−	−	−	+	+	−	|	+	+	−	+	|	−	−	+	+
11.....	−	|	+	|	−	+	+	−	−	|	±	−	−	++	+	−	++	−	−	−	+	±
12.....	−	+	+	|	−	+	+	−	−	+	±	−	−	++	+	−	+	−	−	−	|	+
13.....	−	|	+	+	−	++	+	−	−	|	±	−	−	++	+	−	+	−	−	−	|	±
14.....	−	|	+	|	−	+	|	−	−	−	±	−	−	++	+	−	+	|	−	−	−	±
15.....	−	|	+	|	−	++	|	−	−	−	±	−	−	++	+	−	+	−	−	−	−	±
16.....	−	|	+	+	−	±	−	−	−	−	±	−	−	++	+	−	+	−	−	−	+	±
17.....	−	|	|	|	−	|	−	−	−	−	±	+	−	++	+	−	+	−	−	−	−	±
18.....	−	?	+	|	−	|	−	−	−	−	±	−	|	++	++	−	+	−	−	−	+	±
19.....	−	|	+	|	−	|	−	−	−	−	±	−	?	++	++	−	+	−	−	−	−	±

TABLE V.—Continued.

TION	URINE.			MILK.			ACIDOPHILES ACETIC ACID BROTH.			DIET.	REMARKS.
Type of growth.	Indican.	Indolacetic Acid.	Millon's.	Coagulated.	Gas.	Peptonized.	5 per cent.	10 per cent.	20 per cent.		
+										Bread and bananas.	
+										"	
+										"	
+				+	20	+				"	
+				+	–	+				"	
+				+	–	+				"	
+				+	–	+				"	
+				+	5	+				"	
+				+	5	+				"	
+				+	10	+				"	
++				+	30	+				"	
+				+	–	+				"	
+				+	–	+				Milk and dextrose	
+	–			+	–	+				"	
+	–			+	|	+				"	
+	–			+	–	+				"	
+	–			+	–	+	++	++	+	"	
+	–			+	–	+	++	++	+	"	
+	–			+	–	+	++	++	+	"	
+	–			+	–	+	++	+	+	"	
+	–			+	–	+	++	|	|	"	
+	–			+	–	+	++	++	++	"	
+				+	–	+				"	
+										"	
+										"	
+	–			+	–	+				"	
+										"	
+										"	
+	|	–	–	+	10	+	+	+	+	"	
+	+	–	+							"	
+	–	–	|							"	
+							++	++	+	"	
+										"	
++										"	
++							++	|	–	Eggs.	
++										"	
++										"	
++							+	–	–	"	
++										"	
++	+++	–	+++				+	–	–	"	
++	+	–	++				+	–	–		
++							|	–	–		

TABLE VI.

Monkey III.

DATE.	FECES. GRAM. STAIN.											FERMENTATION TUBE SEDIMENTS. GRAM STAIN.										
	Bifidus.	Acidophilus.	Large subtiloid.	Small subtiloid.	Spores.	Coli.	Cocci.	Long chains.	Short chains.	Spiral forms.	Type.	Bifidus.	Acidophilus	Large subtiloid	Small subtiloid.	Spores.	Coli.	Cocci.	Long chains.	Short chains.	Spiral forms.	Type.
VI- 7	−	+	|	+	−	|	+	−	−	+	±	−	+	|	|	−	|	+	−	−	|	+
8	−	+	|	+	−	+	−	−	−	+	±	−	+	+	|	−	|	++	−	−	+	+
9	−	+	+	|	−	+	+	−	−	+	±	−	+	+	+	−	+	+	−	−	+	+
10	−	+	|	|	−	|	+	−	−	+	+	−	+	+	+	−	+	+	−	|	+	+
11	−	+	|	|	−	−	+	−	−	+	+	−	+	+	−	−	|	+	−	|	+	+
12	−	+	|	|	−	−	+	−	−	+	+	−	+	|	−	−	|	+	−	|	+	+
13	−	+	+	|	−	−	+	−	−	+	+	−	+	|	|	−	+	+	−	|	+	+
14	−	+	|	|	−	|	+	−	−	++	+	−	+	|	|	−	+	+	−	−	+	+
15	−	+	+	−	−	|	+	−	−	+	+	−	+	−	−	−	|	+	−	−	++	+
16	−	+	−	−	−	−	+	−	−	+	+	−	+	−	−	−	−	+	−	−	+	+
17	−	+	−	−	−	|	+	−	−	++	+	−	+	−	+	−	+	|	−	−	+	+
18	−	+	−	−	−	−	+	−	−	+	+	−	+	−	|	−	|	+	−	−	+	+
19	−	+	|	|	−	|	+	−	−	+	+	−	+	|	−	−	−	+	−	−	+	+
20	−	+	−	|	−	+	+	−	−	+	+	−	+	−	−	−	|	|	−	−	+	+
22	−	+	|	|	−	|	|	−	−	+	+	|	+	++	+	−	+	+	−	−	+	±
23	−	+	+	+	−	+	+	−	|	+	±	−	−	++	+	−	+	|	−	−	+	±
24	−	−	+	+	−	+	+	−	+	|	±	−	−	++	+	−	++	+	+	+	+	±
26	−	−	+	+	−	+	|	−	−	|	±	−	−	++	++	|	+	|	−	−	|	±
27	−	−	+	|	|	++	|	−	−	|	±	−	−	++	++	+	+	?	−	−	|	±
28	−	−	+	|	+	+	|	−	−	+	±	−	−	++	+	−	+	|	−	−	|	±
29	−	−	+	|	|	+	|	−	−	|	±	−	−	++	+	−	+	|	−	−	+	±
30	−	−	+	+	−	+	|	−	−	+	±	−	−	++	++	+	−	−	−	−	|	±
VII- 1	−	−	+	+	|	+	|	−	−	|	±	−	−	++	+	−	+	−	−	−	+	±
2	−	−	+	+	|	+	−	−	−	+	±	−	−	++	+	−	+	−	−	−	+	±
3	−	−	+	+	−	+	−	−	−	|	±	−	−	++	+	−	+	−	−	−	+	±
4	−	|	+	+	−	+	|	−	|	|	±	−	−	++	+	−	+	−	−	|	+	±
5	−	−	+	+	|	+	+	−	|	+	±	−	−	+	+	−	|	−	−	−	+	±

TABLE VI.—Continued.

URINE.			MILK.			ACIDOPHILES; ACETIC ACID BROTH.			DIET.	REMARKS.
Indican.	Indolacetic acid.	Millon's.	Coagulated.	Gas.	Peptonized.					
									Milk and Dextrose.	
									"	
									"	
–			+	20	|				"	
									"	
									"	
									"	
–			+	10	+				"	
–			+	5	+				"	
–			+	20	+				"	
–			+	5	+				"	
–			+	10	+				"	
–			+	5	+				"	
–			+	5	+				Eggs.	
|			+	20	+				"	
–			+	20	+				"	
|			+	40	+				"	
			+	95	+				"	
+		+	+	80	+				"	
+		+	+	90	+				"	
+	–	++							"	
++	–	++							"	
+	–	++							"	
+	–	+							"	
+	–	+							"	
+	–	+							"	
									"	

TABLE VII.—Continued.

DATE.	FECES. GRAM STAIN. Bifidus.	Acidophilus.	Large subtiloid.	Small subtiloid.	Spores.	Coli.	Cocci.	Long chains	Short chains.	Spiral forms	Type.	FERMENTATION TUBE SEDIMENTS GRAM STAIN. Bifidus.	Acidophilus.	Large subtiloid.	Small subtiloid.	Spores.	Coli.	Cocci.	Long chains.	
VII-19...	|	+	+	−	+	|	−	−	−	+	+	|	+	−	|	−	−	−	−	−
20...	?	+	|	−	+	−	−	−	−	+	+	|	+	−	|	−	−	−	−	−
21...	−	|	+	+	|	+	|	−	−	+	±	+	+	++	+	−	+	+	−	−
22...	−	−	+	+	|	+	|	−	−	+	±	−	?	++	+	−	+	+	−	−
23...	−	|	+	+	|	+	|	−	−	+	±	−	?	++	+	−	++	+	−	−
24...	−	−	++	+	+	+	|	−	−	+	±	−	−	+++	+	−	+	−	−	−
25...	−	−	+	+	−	+	|	−	−	+	±	−	−	+++	+	−	+	−	−	−
26...	−	−	+	+	−	+	|	−	−	+	±	−	−	+++	+	−	+	−	−	−
27...	−	|	+	+	−	+	+	−	−	+	±	−	−	+++	+	|	+	−		−
28...	−	|	+	+	−	+	|	−	|	+	±	−	−	+++	+	−	+	−	−	−
29...	−	|	+	+	−	+	−	−	|	+	±	−	|	+++	++	−	++	−	−	−
30...	−	?	++	++	−	+	−	−	−	+	±	+	+	+++	++	−	++	+	−	−
31...	−	|	++	+	−	+	−	−	−	+	±	+	+	++	++	−	+	+	−	−
VIII-1...	−	|	++	+	−	+	−	−	−	+	±	+	+	++	++	−	+	+	−	−
2...	−	|	++	+	−	+	−	−	−	+	±	−	+	++	++	−	+	|	−	−
3...	−	+	+	|	−	+	+	−	−	+	+	−	+	++	+	−	|	|	−	−
4...	−	+	|	−	|	−	−	−	−	+	+	−	+	++	++	−	|	|	−	−
5..	−	+	+	+	−	−	+	−	−	+	+	++	+	++	++	−	|	|	−	−
6...	+	+	|	|	|	|	+	−	−	+	+	++	+	++	+	−	+	|	−	−
7...	−	+	+	|	−	|	+	−	−	|	+	++	+	+	++	+	−	+	−	|
8...	+	+	+	+	−	|	+	−	−	+	+	−	+	++	+	−	+	|	|	−
9...	−	+	+	−	+	|	−	−	−	+	+	+	+	++	+	−	+	+	−	−
10...	−	+	+	−	+	−	−	−	−	+	+	−	+	+	|	−	+	|	−	−
11...	−	+	+	+	|	+	|	−	−	|	+	−	+	+	+	−	+	|	−	−
12...	−	+	+	+	−	+	|	−	−	+	+	−	|	+	+	−	+	−	−	−
13...	−	|	+	+	−	+	|	−	−	+	±	−	|	++	++	−	++	−	−	−
14...	−	|	++	+	−	+	|	−	−	−	±	+	+	+++	++	−	++	|	−	−
15...	−	+	++	+	|	+	−	−	−	+	±	−	+	+++	++	−	++	|	+	
16...	−	|	++	+	|	+	−	−	−	+	±	−	|	++	++	+	++	|	+	
17...	−	|	++	+	|	+	|	−	|	+	±	−	−	++	++	−	++	+	−	
18...	−	|	++	+	−	++	+	+	|	+	±	−	|	++	+	−	+	|	−	
19...	−	|	++	+	−	++	+	−	|	+	±	−	+	+	|	−	+	|	−	
20...	−	+	|	|	−	+	|	−	−	+	±	+	++	?	?	−	−	|	+	
21...	−	+	|	|	−	?	|	−	−	++	+	+	++	|	?	−	−		−	
22...	−	+	+	|	−	?	+	−	−	+	+	−	++	?	?	−	−	−	−	
23...	−	+	|	?	−	?	|	−	−	++	+	−	+	+	|	−	|	−	−	
24...	−	+	+	−	−	|	−	−	−	+	+	−	+	+	−	+	|	−	−	
25...	−	+	+	|	−	+	−	−	−	+	±	−	+	++	+	−	+	|	−	
26...	−	|	+	+	−	+	−	−	−	|	±	−	?	++	++	−	+	−	−	
27...	−	|	+	+	−	+	|	−	−	|	±	−	?	++	++	−	+	−	−	
28...	−	|	+	+	−	+	−	−	−	|	±	−	−	++	++	−	++	−	−	
29...	−	?	++	+	−	++	+	−	−	|	±	−	−	++	++	−	++	−	−	
30...	−	?	++	+	−	++	+	−	−	|	±	−	−	++	++	−	++	−	−	

TABLE VII.—Continued.

ON	URINE.			MILK.			ACIDOPHILES ACETIC ACID BROTH.			DIET.	REMARKS.
Type of growth.	Indican.	Indolacetic acid.	Millon's.	Coagulated.	Gas.	Peptonized.	5 per cent.	10 per cent.	20 per cent.		
+											
+				++	|	+	++	++	+	Eggs	
++	++	−	++	++	90	+	++	++	+	"	
++	++	−	++				++	|	|	"	
++	++	−	++							"	
++	++	−	++				++	−	−	"	
++	+	−	+							"	
++	++	−	++	+	100		+	−	−	"	
++	+	−	++	+	100	+				"	
++	+	−	+							"	
++	+	−	+	+	40	+				"	
++	|	−	|	+	20	+				"	
+										"	
+										Milk and dextrose	
+										"	
+	−	−	−							"	
+	−	−	−							"	
+	−	−	−							"	
+										"	
+	−	−	−							"	
+	−	−	−	−						"	
+										"	
+										"	
+										"	
+										Eggs	
++	+	−	+							"	
++										"	
++	+	−	++							"	
++										"	
++	+	−	+							"	
++	+	−	+							"	
++										"	
+	+	−	+							"	
+										"	
+	|	−	+							"	
+	−	−	−							"	
+	|	−	|							"	
++										"	
++	+	−	++							"	
++										"	
++										"	
++	++	−	++							"	
++										"	

EXPLANATION OF THE PLATES.

PLATE I. Fig. I. Protein diet. Monkey. Feces. The Gram-stained fields show mixed Gram-positive and Gram-negative flora. The former consists to a considerable degree of large and small subtiloid bacilli, with a few coccal forms. The latter is composed largely of organisms referable morphologically and culturally to *B. coli* and its variants. *1* and *2*, large subtiloid organisms; *3*, *B. coli*; *4*, small subtiloid bacilli.

FIG. II. Protein diet. Monkey. Dextrose sediment. The morphological differences are br[illegible]ut more clearly, due in all probability to the fact that the o[illegible]s are in active vegetative development. *1* and *2*, large subti[illegible]cilli; *3*, *B. coli*; *4*, small subtiloid bacilli.

PLATE II. Fig. III. Carbo[illegible] diet. Monkey. Feces. The fields differ from those of the p[illegible]t, being much more homogeneous, both with respect to the st[illegible] d to the morphology of the organisms. The most prominent [illegible]presented is *B. acidophilus*, with, however, a moderate number [illegible]*fidus*. It is not possible to differentiate with certainty between these two organisms, morphologically, unless one sees them in artificial media.

Fig. IV. Carbohydrate diet. Monkey. Dextrose sediment. This sediment is less characteristic than many derived from this source, but is introduced to show the morphology of those organisms characteristic of this diet. *1*, *B. acidophilus*, long curved form; *2*, *B. acidophilus*, shorter form (this organism is more slender than the large subtiloid bacillus, and longer than a small subtiloid organism); *3*, curved form of *B. acidophilus*; *4*, typical *B. bifidus*.

PLATE III. Fig. V. Transitional stage from carbohydrate to protein diet. Monkey. Feces. The organisms, with the exception of the large forms, are undergoing a granular degeneration. This is characteristic of the appearance of the Gram-stained feces during that period elapsing from the disappearance of the carbohydrate flora to tbe establishment of the protein flora. The organisms diminish in size, and stain irregularly. *1*, degenerating acidophiles; *2*, subtiloid bacillus.

Fig. VI. Transitional stage. Dextrose sediment. Monkey. The degenerative character of the acidophiles is shown. The organisms develop slowly, and are atypical morphologically and in staining reaction. *1* and *2*, *B. bifidus*; *3*, subtiloid bacilli; *4*, degenerating acidophilic bacteria.

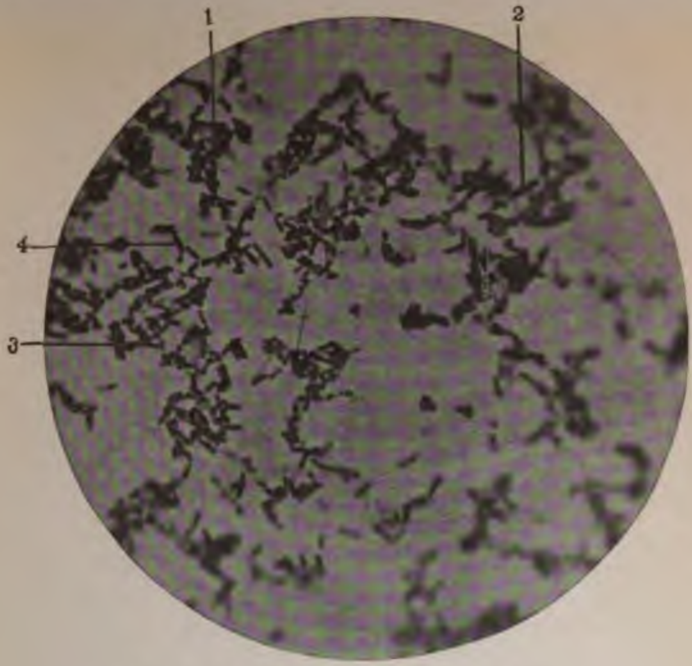

FIG. 1. PROTEIN DIET: FECES. GRAM STAIN.

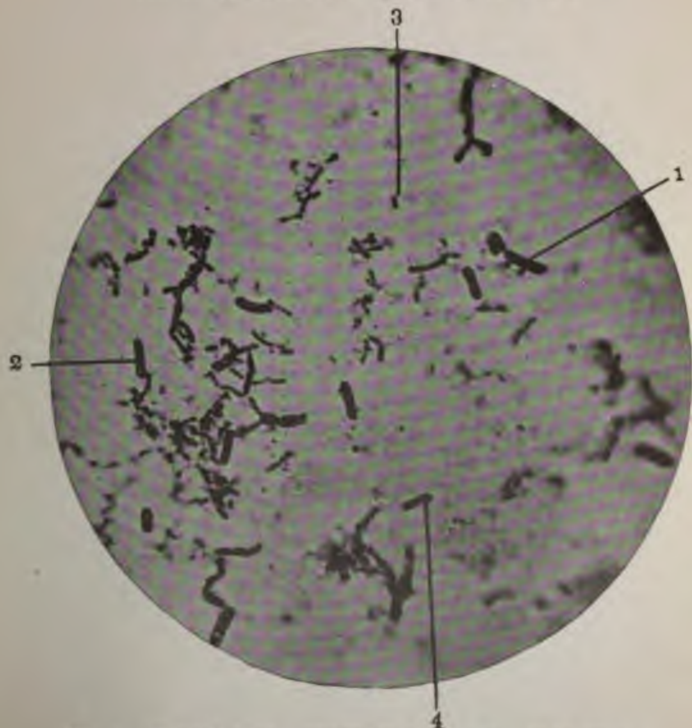

FIG. 2. PROTEIN DIET. DEXTROSE SEDIMENT. GRAM STAIN.

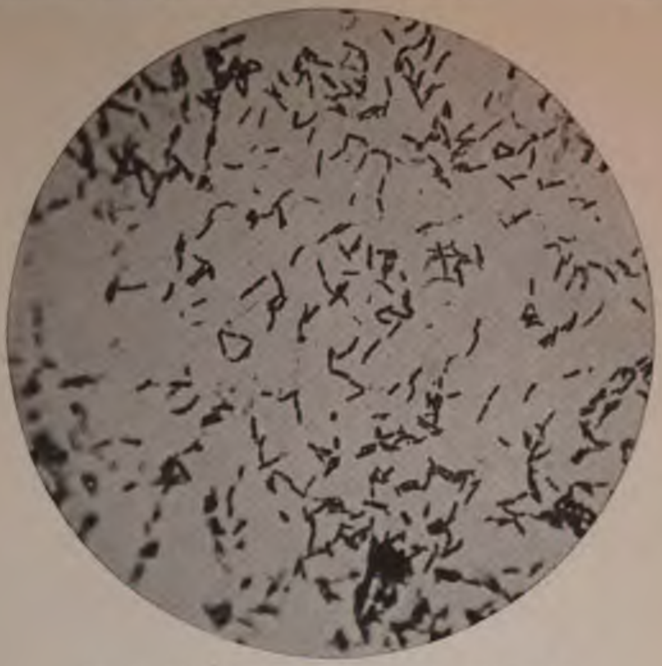

FIG. 3. CARBOHYDRATE DIET: FECES. GRAM STAIN.

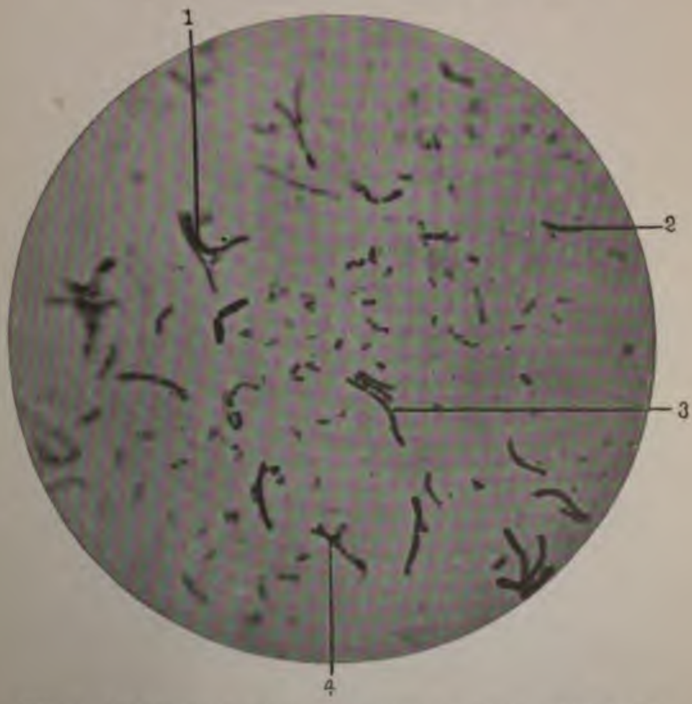

FIG. 4. CARBOHYDRATE DIET. DEXTROSE SEDIMENT. GRAM STAIN.

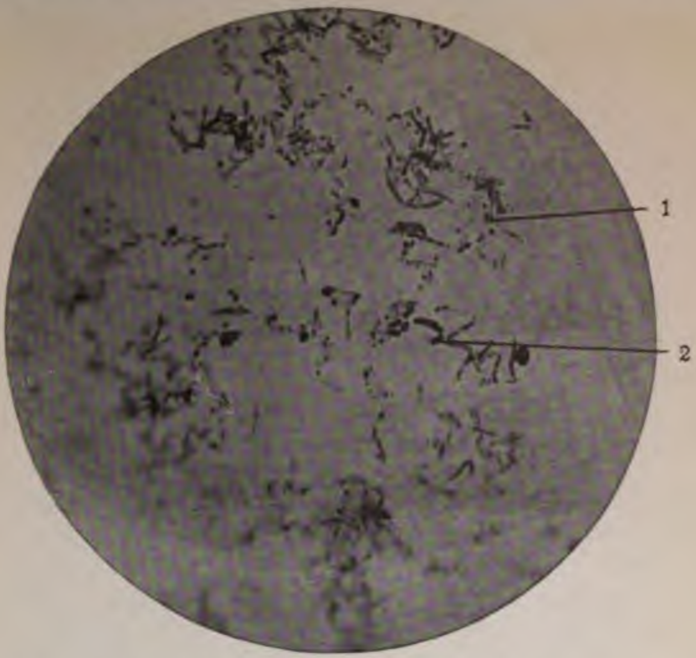

FIG. 5. TRANSITIONAL STAGE: CARBOHYDRATE TO PROTEIN. FECES. GRAM STAIN.

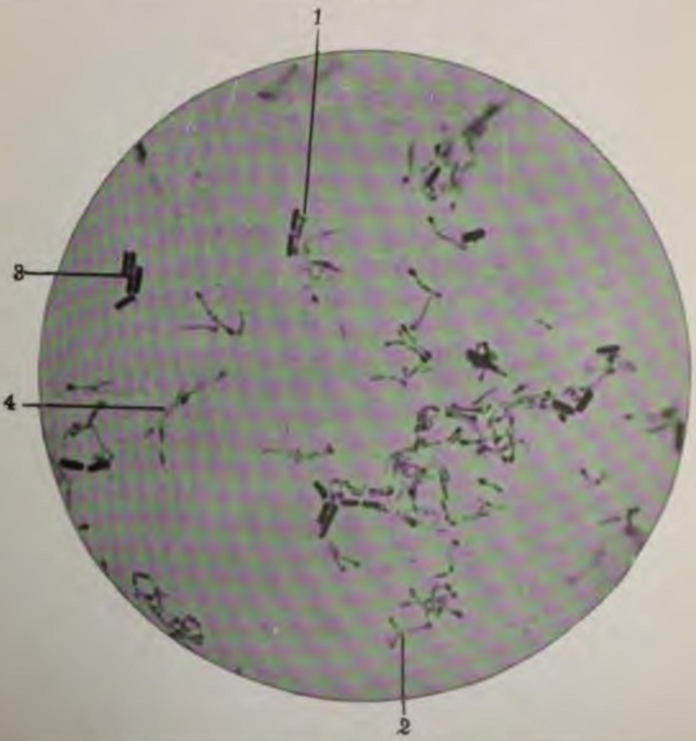

FIG. 6. TRANSITIONAL STAGE: CARBOHYDRATE TO PROTEIN DIET. DEXTROSE SEDIMENT. GRAM STAIN.

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THE ACTION OF SODIUM BENZOATE AND BENZOIC ACID ON THE HUMAN ORGANISM

BY

C. A. HERTER

U. S. REFEREE

Professor of Pharmacology, Columbia University,
Consulting Physician City Hospital

This paper was offered to the Journal of the American Medical Association for publication, but was refused by the Editor, partly on account of its length, partly because the Journal did not care to open its pages to "anything more of a controversial nature regarding the benzoate question," partly because the criticisms of Bulletin No. 84, contained in this paper, were regarded as impolitic as coming from the Journal.

Deeming it important to a correct understanding of the benzoate question that physicians should be acquainted with the facts given in my paper I have decided to privately print it and distribute it among members of the medical profession.

CHRISTIAN A. HERTER.

May 10, 1910.

THE ACTION OF SODIUM BENZOATE AND BENZOIC ACID ON THE HUMAN ORGANISM.

By C. A. HERTER.
U. S. Referee.
(*Professor of Pharmacology Columbia University, Consulting Physician City Hospital.*)

Introductory.

Since the publication of the Report of the Referee Board[1] on the action of sodium benzoate, this subject has been widely ventilated in the newspapers and magazines, though little discussed by medical men. Believing that a pharmacological subject with so many technical phases cannot be satisfactorily presented and adjudicated by the lay press, I desire to lay the physiological properties of sodium benzoate before the medical profession, as it is only here that training exists adequate to reach a scientific conclusion.

It will be remembered that the questions put by the Secretary of Agriculture to the Referee Board had to do with the harmfulness or harmlessness of sodium benzoate as a food preservative.

The conclusions of the Referee Board were expressed as follows:

1. Sodium benzoate in small doses (under 0.5 gram per day) mixed with the food is without deleterious or poisonous action and is not injurious to health.
2. Sodium benzoate in large doses (up to 4 grams per day) mixed with the food has not been found to exert any deleterious effect on the general health, nor to act as a poison in the general acceptation of the term. In some directions there were slight modifications in certain physiological processes, the exact significance of which modifications is not known.
3. The admixture of sodium benzoate with food in small or large doses has not been found to injuriously affect or impair the quality or nutritive value of such food.

---

[1]The Influence of Sodium Benzoate on the Nutrition and Health of Man. U. S. Department of Agriculture, Report No. 88. May 4, 1909.

Although these conclusions have been widely attacked in
lay press and to some extent in the medical journals, few rec
writings of a scientific nature bear on them. At the time of
issue of the Report of the Referee Board (Bulletin No. 88) th
existed a Report (Bulletin No. 84) of the United States Depa
ment of Agriculture written by Dr. H. W. Wiley and his co
borators dealing also with the action of sodium benzoate.[1] As
conclusions expressed in this report are in most respects qu
opposed to those of the Referee Board it is only natural that opp
ing opinions and convictions should have rallied about these t
reports. Recently there has been published a strongly bia
paper dealing with the action of benzoic acid on animals and m
in which experiments are reported which claim a somewhat sta
ling grade of toxicity for benzoic acid. If we exclude various d
tribes of a general nature which make not even a superficial p
tense to scientific merit, we find that the two communications j
mentioned (Bulletin No. 84 and the paper by Lucas) furnish
opponents of the Referee Board with the chief materials for th
endeavors to discredit its conclusions. I believe I shall be able
the following pages to show that in the benzoate question the
parently opposing views are in reality not of equal accuracy a
merit. I purpose to show that the Report of the Referee Boa
has not been disproved in any particular and that its conclusio
remain unshaken by the controversial assaults upon them.
purpose, also, to show that those who are on record with conc
sions opposed to those of the Referee Board have committed
series of scientific blunders which will open the eyes of any unp
judiced student of the question to the fact that the apparent e
dence against the conclusions of the Referee Board is of a natu
so flimsy as to completely break down under searching criticis

[1] WILEY, H. W., with the collaboration of W. D. Bigelow, F. C. Weber a
others. Influence of Food Preservatives and Artificial Colors on Dig
tion and Health. IV. Benzoic Acid and Benzoates. United States Depa
ment of Agriculture, Bureau of Chemistry. Bulletin No. 84, Part iv,
1043-1294, 1908.

[2] LUCAS, DANIEL R., M.D. Some Effects of Sodium Benzoate. *Jo
Amer. Med. Assn.*, liv, p. 759, 1910 (March 5, No. 10).

I propose to discuss this subject under the following heads:

1. Plan of experiments and mode of administering benzoates and benzoic cid.
2. Clinical effects of benzoates and benzoic acid.
3. Effects on digestive conditions.
4. Effects on metabolic conditions.
5. Effects on the kidneys.

1. Plan of Experiments and Mode of Administration.

In any elaborate study of a pharmacological question the plan of procedure is of the first importance, but it does not follow that all investigators of the same question should follow exactly the same plan. The three experiments of the Referee Board on benzoate of soda were not identical. They cover a wide territory and are by far the most extensive and carefully conducted experiments that exist on this subject. Few criticisms which can by any stretch of courtesy be called valuable have been received by the Referee Board, but a great variety of rather thoughtless comment has been made. The Referee Board has been criticized for not carrying on its experiments over a much longer period of time; for not extending them to sick people and babies; for not carrying out certain clinical observations, such as frequent records of the blood pressure. The experiments from my laboratory have been criticized because the subjects (who were also analysts) did not take their pulses, temperatures, respiration and blood pressures several times a day in addition to collecting their perspiration. These details, which I consider of secondary importance, were purposely dispensed with in my series because their observance would have seriously interfered with the work of the subjects and would have had a detrimental effect in calling undue attention to their own condition. In the set of experiments carried out by Professor Long, which were in some respects less exacting, the pulse, temperature and respiration were carefully noted, so that these points have not, in reality, been overlooked by the Referee Board.

As regards the duration of our experiments I would say emphatically that studies of this nature cannot be indefinitely carried on, as they call for a degree of coördination and an exacting attention to a multiplicity of details which after a time induce an *ennui*

which makes continuance increasingly difficult. This is a point which can only be appreciated fully by those who have undertaken an elaborate investigation of this sort. In reality our experiments on benzoate lasted long enough to reveal any poisonous effects—did such pertain to the doses used—since there is not the shadow of evidence that the benzoates have any cumulative effect, like digitalis or arsenic. Moreover they were of longer duration than any others that have been made. The amounts administered were also large enough to fully test the question of injurious action, *having been far above any possible quantities that would ordinarily be ingested as a preservative.* The difficulties of studying the effects of benzoate on sick people and babies[1] are very obvious, and it is equally clear that such an undertaking cannot be regarded as essential to answer the questions asked by the Secretary of Agriculture. Any injurious effects of the substances in question could be much more clearly and precisely observed in normal persons than in persons whose functions are modified by disease. The mode of administration deserves a word. *The object in such an experiment should be to obtain conditions as closely similar as possible to the conditions that would exist if preserved food were taken.* Now it is quite clear that such conditions cannot be perfectly imitated. In order to give, say, three grams a day of sodium benzoate in a concentration of one-tenth of one per cent, (that most commonly employed) it would be necessary to administer three litres of benzoated food in a day. And as such food is mostly of vegetable nature, such as fruits, apple-butter, catsups, etc., it is evident that it would be absurd to expect to give such large doses in the form of preserved food without increasing the concentration of the benzoate far beyond that employed in practice—a manifestly unfair condition. In the experiment of the Referee Board, benzoate was added to the food and naturally for the most part to the fluid constituents of the food, such as soup, milk, water, etc. My experiments have been criticized because some of the benzoate was added to milk. The use ef benzoate in milk, however, was simply part of what seemed the necessary practice of giving the benzoate in fluid or semi-fluid food, as it could not so easily be evenly distributed in solid food.

[1] Babies have been shown to be very tolerant of even relatively large doses of sodium benzoate.

It should be remembered *that benzoate when thus given in various fluid or semi-fluid foods would not remain confined to these in the stomach but would tend to become generally diffused through the semi-fluid contents of the stomach.* It is difficult to see how any fair-minded critics can object to such a method of administration.

On the other hand to administer sodium benzoate or benzoic acid in a concentrated form, so that there is a probability of actual contact of the crystalline substance with the walls of the stomach or intestine, is so remote from what happens in the use of any preservative as to be a glaring artificiality. Such a method must be designated an experimental blunder. This, however, is the method which was regularly and continuously employed in the experiments reported in Bulletin 84, for here benzoate and benzoic acid were given in solid form in capsules (presumably gelatin) so that the stomach was liable to be first exposed to the utmost concentration of these substances that was attainable in the fluids into which they were diffused after solution of the capsule.

2. Clinical Effects of Administration of Benzoate of Soda and Benzoic Acid.

One might safely predict the occurrence of disturbances of digestion after giving these substances in capsules. How could it be otherwise? Everyone knows that the liberation of free strong acids exerts injurious effects on all living tissues, and no method could have been more effectively designed to injure the digestive tract by means of benzoic acid or benzoates. The large number of digestive derangements reported in Bulletin No. 84 can probably be fairly referred to this method of administration. Surprising as it may seem, however, a still more ingenious method of giving sodium benzoate has been used by Lucas, by whom dogs were fed relatively enormous doses of sodium benzoate (about 1 gram to the kilo) from which the acid was promptly liberated by giving large doses of hydrochloric acid.[1] It cannot even be pretended that such

[1] Exactly the same bias that is shown in the Lucas paper in the experiments on human beings is shown in the experiments on dogs. The following extraordinary experiment is reported: A fasting dog weighing 3.5 kilos received sodium benzoate and hydrochloric acid in a preliminary experiment. The day after recovery from muscular weakness, nausea, etc., it

extreme experiments throw any light on the action of sodium benzoate on the human body, since the conditions of administration are grotesquely remote from anything that ever happens when benzoates are used in food. Would such experiments be introduced in an unbiassed discussion of the effects of benzoates in preservatives?

It has been intimated by a sensational magazine writer that the experiments of the Referee Board prove that there were great disturbances of digestion due to the sodium benzoate which the

received 4 grams of sodium benzoate in an amount of hydrochloric acid theoretically sufficient to decompose it completely to free benzoic acid, together with 100 c.c. of a two per cent. citric acid. The animal became very weak, developed convulsions and died soon after the dose, showing congestion, ulceration and hemorrahage, etc., in the digestive tract, liver and lungs. Anyone who will reflect on what this experiment represents in the way of benzoic acid in a human being will see that it is equivalent to giving 80 grams of benzoate of soda to a man weighing 70 kilos. The effect in such an experiment is to liberate benzoic acid in its most concentrated form to say nothing of free solid benzoic acid. How any other outcome than gross damage to the digestive tract was to be expected it is difficult to see, the results obtained being merely in accord with what every pharmacologist knows to be the action of strong acid. The fact that dogs receiving hydrochloric acid and citric acid in considerable quantities without benzoic acid did not become sick and die, presents no unexpected features. It is doubtful if any competent and fair-minded investigator would put forward such an experiment as the one quoted above as an argument in regard to the action of benzoic acid as a food preservative.

I am constrained to refer again to the prejudiced spirit of the Lucas paper, which shows an ill-concealed tendency to make out the very worst possible case against benzoate of soda, regardless of the facts. When the unintelligent experiments on dogs were read at Denver the terrifying effects of the substance were given in detail. One of the dire consequences of poisoning by benzoic acid was "that the liver and lungs *showed evidence of infarcts*" in the first experiment. In a second experiment also "the liver and lungs showed considerable congestion *with some evidence of infarcts*." (The italics are mine.) The anti-benzoate enthusiasm needful to picture this unlikely development of infarcts appears to have subsided in the atmosphere of circumspection and truthfulness which prevails in New York, and in the published account of the experiments the "infarcts" have been judiciously converted into mere hemorrhages. Every pathologist can see that the reported protocols do *not* point to hepatic and pulmonary infarcts and some adumbration of this must have reached the parents of the paper, on whom rested the painful burden of bringing the anatomical proofs which are wholly absent in the published descriptions of the lesions.

members of the Board either were not intelligent enough to recognize as such or deliberately overlooked. A startling showing is made by extracting from the notes on sixteen men every digestive symptom and listing them quite dislocated from their context. The following facts were entirely overlooked: namely, that the disturbances referred to were slight, occurred during the trying summer months when people are more than ordinarily subject to slight digestive disorders, and that competent physicians were accessible to scrutinize the meaning of such subjective and objective disturbances as were reported by the subjects of the experiments.[1]

It is noteworthy that our men had a sense of well-being during the greater part of the experiments and quitted them in the best of health, having gained in weight during the benzoate period with one exception, where there was no loss. In case of the experiments reported in Bulletin No. 84 there was a loss in weight which

[1] In my experiments Dr. John S. Thacher was the physician in charge. He is a man distinguished for fairness of mind, experience and good judgment—a man with a quarter of a century of experience in scientific laboratory methods and now a distinguished practitioner and attending physician to the Presbyterian and Roosevelt Hospitals. We are asked, however, to lay aside the views of this physician as to the significance of the slight disturbances which occurred in our group of men and to prefer the interpretation placed upon them by a scientifically uneducated and irresponsible magazine writer and by a young physician,—a subject in our experiments—who had shown a disregard for the reasonable regulations under which the experiments were conducted and who has recently published his own interpretation of the value of the slight disturbances observed by him when taking large doses of sodium benzoate. Dr. Lucas reported to Dr. Wakeman (immediately in charge of the laboratory) on one occasion that he experienced some uneasiness after taking prune juice to which he had added sodium benzoate. But aside from this Dr. Wakeman cannot recall any report bearing on this subject. Dr. Wakeman says "I am unable to find in Dr. Lucas's note-book in which he entered his symptoms any reference to any other effects from taking sodium benzoate in acid portions of the food." The addition of benzoate to acid portions of the food taken at the beginning of some of the meals was a procedure carried out by Dr. Lucas on his own initiative. As he was taking 2.5 grams of sodium benzoate daily there was here an opportunity for the liberation of a considerable quantity of benzoic acid in concentrations far in excess of that employed in any preservatives. That some symptoms of gastric irritation should have arisen under these circumstances so far removed from the conditions under which benzoated food would ordinarily be taken, is not surprising.

is attributed to the poisonous action of sodium benzoa
interpretation is difficult to support but it is not imposs
owing to the method of administration of sodium benzo
disturbance of absorption was induced, detrimental to the
of weight. Possibly underfeeding contributed to the res

The careful observations of the Referee Board relatin
clinical examination of the blood failed to show any de
effects from the administration of sodium benzoate in
employed.

3. Effects on Digestive Conditions.

In the report from my laboratory several slight mod
of function were noted in relation to the digestive tract
these was a rise in the free hydrochloric acid of the gas
which showed a tendency to be secreted in quantities ap
the upper physiological limits. *It is noteworthy that thi
tion of gastric secretion was not associated with any observa
toms such as are often associated with pathological hyperch*
The most significant feature of the rise of the hydrochlo
the fact that *it may be regarded as affording the very best o
ties for the extensive liberation of benzoic acid from the so
zoate ingested, thus giving the fullest opportunity for an
action which the liberated benzoic acid might exert.* This fa
it especially noteworthy that during the high benzoate pe
were no observable signs of gastric irritation.

A slight increase in the indican of the urine was noted durin
benzoate period, but the rise was so slight that it might easily ha
detection. I have been inclined to attribute it to a slight stimul
tative action on the lower part of the small intestine and perhaps
part of the colon. But at most it is possible to attach to it only sl
ficance. Such significance as it possesses I should regard as poin
unfavorable action on digestion from the continuous and prolon
large doses of sodium benzoate. The depression of the gas-for
tions of the mixed fecal bacteria I am unable to satisfactorily
This phenomenon has been wholly misunderstood by some critic
... No. 88, for they have assumed that this depression in gas
... to the gas formed within the intestine itself, whereas m
... to do solely with gas phenomena observed in fermenta

The moderate rise in the proportion of coccal bacteria obser
... tube sediments after inoculation with the mixed

I am also unable to satisfactorily interpret and the results were reported only because it did not seem fair to arbitrarily ignore them in an impartial report. Possibly this phenomenon points to slight irritation through the action of large doses of sodium benzoate but I do not think this view can be positively maintained. It was my impression, however, that on the whole the slight modification of physiological function in the digestive tract which I have referred to could best be accounted for by supposing that the gastro-enteric mucous membrane in some part of its course had been subjected to a slight stimulant or irritative action and that this action was exerted by the continued use of rather large doses of sodium benzoate—doses much above the amount which would be ingested by even the very free use of benzoated foods.

In Bulletin No. 84 it is stated that there was a diminished absorption of food from the alimentary canal, not during the benzoate of soda period, but *after* the withdrawal of the substance. *No reason except accidental association is given for connecting this decreased absorption with the ingestion of sodium benzoate.* The report made in Bulletin No. 88 from the Referee Board is based on accurate and adequate methods of determining the absorption of proteins and fats. *The results show in the most definite way that the taking of benzoate of soda, even in large doses, over a considerable period of time, caused no interference whatever in the extent of absorption of these highly important food-stuffs, during either the benzoate period or the after period.*

Let us turn for a moment to the consideration of the effect of benzoic acid on the digestive enzymes. The statement is made in the Lucas paper that a subordinate relation to benzoic acid is shown by sodium benzoate when comparative toxicity to other enzymes and bacteria is considered. This remarkable claim is, perhaps, as illuminating as the assertion that common salt is less toxic than muriatic acid! Certain numerical results, carefully selected from a table found in T. Lauder Brunton's text-book[1] (but not based on Brunton's own work) are reproduced apparently with the idea of giving the impression of great toxicity for benzoic acid. For example, emulsin is said to be inhibited by 1:2100 benzoic acid. As a matter of fact, however, if the toxicity of benzoic acid be compared with that of other carboxylic acids mentioned in the table—and surely this is the only fair basis for a comparison—a very different impression is obtained. Thus salicylic acid

[1] Text-book of Pharmacology, Therapeutics and Materia Medica, London, 1878, Ed.3, p. 78.

and sulphurous acid are shown to be three and one-half to ten times as toxic as benzoic acid as regards emulsin. But it is not worth while to discuss the table in detail since the results are very inaccurate. For example, the statement is made and included in the Lucas paper that peptic digestion is easily arrested by benzoic acid in the proportion of 1:200. *This is an entirely erroneous statement, for peptic digestion may proceed actively under suitable conditions in a solution completely saturated with benzoic acid,*[1] although naturally here the velocity of the reaction is diminished. Tryptic Digestion is uninfluenced by the presence of one-tenth per cent sodium benzoate. Likewise it is untrue that emulsin is inhibited by benzoic acid in concentrations of 1:2100. It is thus evident that there exists no foundation for ascribing to benzoic acid any specific inhibiting action on peptic digestion.

4. Metabolic Conditions.

In Bulletin No. 84 considerable weight is laid on the study of the nitrogen balance and it is claimed that the average data for eleven men showed an increase of two per cent. in the preservative period of the amount of ingested nitrogen excreted in metabolized form, "*indicating a tendency to increase to this extent the catabolic functions while the increase of nitrogen in the fæces point to a decrease in nitrogen assimilation.*" It would be impossible and beside the mark to discuss here the question of nitrogen metabolism in extenso, for the reason that ample space would be required to adequately present a subject about which chemical physiologists differ so widely. I do not attach pathological significance to slight variations in the nitrogen balances obtained either by the methods used for the data of Bulletin No. 84 or for the data of Bulletin No. 88; I venture to make the unconventional statement that it is impossible from such experiments to derive any pathological significance from the slight or moderate variations in the nitrogen balances, since the experimental conditions present difficulties so great as to make it unsafe to draw such significant conclusions. The fact that unjustifiable conclusions have been drawn by eminent writers on this subject does not deter me from expressing my conviction on this point.

Bulletin No. 84 takes no account whatever of the partition of the

[1] This subject has been most carefully and fully investigated under my direction by a highly skilled physiological chemist, Mr. H. D. Dakin.

nitrogen in the urine—a consideration of far greater importance than the mere nitrogen balance. Bulletin No. 88, on the other hand, gives the results of detailed studies of the nitrogen of urea, the nitrogen of ammonia, the nitrogen of uric acid, the nitrogen of the purin bases and the nitrogen of creatinin. Particularly important in the benzoate study is the possibility of the occurrence of acid intoxication from benzoates and benzoic acid. The results of the study of the ammonia excretion show that there is not even the shadow of ground for the view that such intoxication existed even in the slighest degree at any time in the experiments made by the Referee Board. Nor did the other nitrogenous constituents show indications of disturbed nitrogen metabolism.

I wish to insist on the significant fact that any disturbance of the nitrogen balances indicative of deranged intermediary nitrogenous metabolism would certainly have revealed itself in some abnormality of the nitrogen partition.

Under metabolic disturbances we may include derangements characterized by the occurrence of reducing substances in the urine. The tables in the Lucas paper give the impression that when benzoated cider is ingested it is followed by the appearance of reducing substances in the urine attributable to the benzoic acid. It is unfortunate that these tables show no adequate controls based on the action of cider alone. I wish to state that unbenzoated cider (two litres in the course of the day) in some persons gives rise to a marked increase in the reducing substances of the urine. For this reason it is wholly unfair, on the strength of the data given, to implicate benzoic acid in the causation of the reducing action noted. It is well known that large doses of benzoate of soda (10 to 20 grams daily) cause the appearance of increased reduction in the urine due to glycuronic acid. Somewhat smaller doses may sometimes suffice to do this. The relatively small quantities taken in benzoated cider do not suffice to produce this effect. Thus we have here another instance of a blunder of interpretation based on unscientific methods of procedure.[1]

[1] An accurate comparison of the varying reducing power of the urine with Fehling's solution can only be obtained by the most careful experiments in which definite volumes of Fehling's solution and of urine of definite specific gravity are employed and boiled for the same length of time, the results being recorded after a definite lapse of time. None of these essential precautions are mentioned in the paper referred to.

5. Effects on the Kidneys.

Perhaps the most notable infelicities connected with Bulletin No. 84 and with the Lucas paper are to be found in the discussion of the effect of benzoate of soda and benzoic acid upon the kidneys. I will briefly review the points at issue under the following heads:

(a) Excretion of hippuric acid
(b) Formed elements of the urine
(c) Albuminuria

a. We learn from Bulletin No. 84 (page 1287) that "hippuric acid is the most important natural constituent of the urine of herbivorous animals, whose food contains large quantities of aromatic substances which, either by oxidation or reduction, are converted into bodies containing the benzene nucleus." Has this sentence any meaning whatever or any relation to fact? How aromatic substances can be converted, either by oxidation or reduction, into bodies containing the benzene nucleus must, I think, always remain a chemical mystery. In the next sentence we are told that "the benzene nucleus by combination with glycocoll is converted into hippuric acid, in which form it is excreted." This performance on the part of the benzene nucleus will be greeted with admiration by all who know its properties. With this turbid introduction to the physiology of hippuric acid, so typical of the entire discussion, we are led to a discussion of the physiology of of hippuric acid formation and excretion. The experiments in Bulletin No. 84 are said to show that when benzoic acid was given, an amount of hippuric acid was recovered corresponding to 81.32 per cent. of the total quantity ingested, while only 61.41 per cent of benzoic acid was recovered as hippuric acid after sodium benzoate had been taken. "*Thus there is shown a marked tendency to restrict the excretion of benzoic acid when administered as benzoate of soda, the total decrease being almost exactly 20 per cent. as compared with the excretion of benzoic acid.*" Then comes the inevitable damaging conclusion. "This fact is another confirmation of what is shown in so many other instances from this study of the retarded effect of the preservative on the system when administered as benzoate of soda."

This conclusion on the part of the writers of Bulletin No. 84

is wholly erroneous for the reason that the data on which it is based are incorrect. It is not true that a large proportion of the benzoic acid introduced as benzoate of soda fails to be excreted as hippuric acid. The work of Lewinski[1] has shown that all the benzoic acid introduced as sodium benzoate is recoverable as hippuric acid where quantities are used such as were recorded in the benzoate experiments reported in Bulletin No. 84 and Bulletin No. 88. In order to test this question independently I asked Dr. Dakin[2] to make a special series of experiments in which the factor of diet was taken most carefully into account. These experiments, which were conducted with great care, show that essentially all of the benzoic acid introduced as sodium benzoate is recoverable as hippuric acid, although as much as ten grams of sodium benzoate a day was ingested in one observation. *In these experiments there was no guesswork as to the substance actually recovered. The hippuric acid was recovered as chemically well defined hippuric acid possessing the various properties of this substance.*

I therefore state that benzoate of soda taken in the amounts given in the experiments recorded in Bulletin No. 84 and Bulletin No. 88 is almost wholly recoverable as hippuric acid and that benzoate of soda in nowise differs from benzoic acid in respect to this recoverability.[3] Furthermore I wish to say that the statement of Bulletin No. 84 that free benzoic acid passes into the urine after the ingestion of benzoate of soda is, in general, false for moderate quantities of benzoate ingested, and is based on incompetent chemical analyses. The alleged benzoic acid was not identified as such and until chemical proof is advanced the negative conclusions of competent physiological chemists must be accepted.

b. Formed elements of the urine. In Bulletin No. 84 considerable attention is paid to the microscopical elements of the urine

[1] Ueber die Grenzen der Hippursäurebildung beim Menschen. *Arch. f. exper. Path. u. Pharm.*, lviii, p. 397, 1908.

[2] The Fate of Sodium Benzoate in the Human Organism. *Jour. Biol. Chem.*, vii, p. 103, 1910.

[3] Bearing on the results obtained by the writers of Bulletin No. 84 is the description of the method employed in determining hippuric acid (See Bulletin No. 84, pages 1046 and 1050). Here it is stated that the urines were "made alkaline" and were "shaken out alkaline." The necessity for acidifying the extracted material was apparently wholly overlooked. Possibly the poor results for hippuric acid are connected with this blunder.

including crystalline substances, epithelial cells, leucocytes, casts, cylindroids, etc. An extraordinary method of recording the results obtained is here employed. Microscopical counts are made of the various bodies in question and are recorded by an arbitrary numerical scale according as there are none, very few, few, many, etc., present. These definite figures are then added and presented as indicating the relative numbers of the formed bodies present in the fore period, the preservative period, and the after period! The precaution is even taken to carry out the figures to two decimal places! Thus the relative occurrence of mucous cylindroids was 141.67, 205.71 and 152.17 for the different periods. Such an assumption of accuracy is wholly misleading and unscientific, when we consider the data on which such numerical comparisons are based. The report goes even further in this ingenious direction and groups together all the different kinds of microscopical bodies of the urine with the statement that the relative occurrence in the three periods was 64.4, 75.2 and 59.1. *In this enlightening report an oxalate crystal has the same value as a pus cell or cast! Under this flimsy method the presumption is set up of an injurious action during the preservative period.* Our observations give absolutely no basis for such a conclusion.

c. Albuminuria. In the Lucas paper we find records of the experiments made with acid fruits and cider. Benzoate of soda was added in various proportions to the acid fruits and to the cider, nearly always in proportions far in excess of any that would be justified for preservative purposes. The alleged object of these experiments was to permit the action of the acid fruits and of the cider to set free the benzoic acid from the sodium benzoate in order to determine the effect of the free acid as distinguished from its sodium salt. Large quantities of cider were taken at a time. In addition to various disorders of digestion, albuminuria was reported and the inference was drawn that benzoic acid acts harmfully upon the kidneys.

The view that free acids are more toxic than their neutral salts has long been a common-place of toxicology and there is nothing surprising about the fact that benzoic acid is both more effective as a preservative and as an injurious agent to protoplasm than sodium benzoate. In the Lucas experiments quantities of benzoic acid were employed so far in excess of the quantities that would

ordinarily be ingested as a preservative, that it is difficult for an unprejudiced student of the question to see what bearing such experiments can have except in determining the limit of toleration That there is a concentration and dose in which benzoic acid has. an injurious irritant action, no one will deny. The investigations of benzoic acid in my laboratory have yielded different results to those reported in the Lucas paper. They show that when considerable quantities (several grams) of sodium benzoate are taken in a strongly acid medium for several days there is usually no sign of albuminuria. In one instance a trace of albumin appeared from time to time, during and between the benzoate periods. There was no evidence of cast formation. In this case an expert cystoscopic examination showed that this abnormality was referable to an irritative condition in the posterior urethra. I have not yet been able to find that renal albuminuria results from the use of moderate doses of sodium benzoate or benzoic acid. Even in a case where albuminuria is generally present, the taking of one gram of benzoic acid in 750 c.c. of water in ten minutes on an empty stomach failed to be followed by the appearance of albuminuria, although it had been present a few days before and reappeared again after the lapse of several days. The ability of the organism to neutralize ordinary doses of benzoic acid with alkali is unquestionable. It is well known that benzoic acid was formerly used freely in bladder disease without causing injury to the kidney. The injurious effects of ingested benzoic acid, like other acids, fall on the digestive tract. For this reason high concentrations of the acid (above 0.1 per cent.) should be avoided, especially when other acids are ingested at the same time.

The readiness with which (according to Lucas) albuminuria follows the taking of benzoate in acid media, is also quite at variance with unpublished experiments made by Dr. Sherman of Columbia University, and by Dr. E. E. Smith. They are also in opposition to the recent careful and extensive work of Gerlach[1] on the action of sodium benzoate and benzoic acid. Nevertheless I deem it inadvisable for some persons to take several grams daily of benzoic acid in an acid medium, on account of the local irritant effect, and this caution holds for acids in general.

[1] Physiologische Wirkungen der Benzoesäure und des benzoesauren Natron. Heinrich Staadt, Wiesbaden, 1909.

FURTHER COMMENTS.

In the Lucas paper a wholly erroneous impression is f to the relation between the preservative powers of be soda and its toxic action when given in acid food. One p benzoate of soda is said to be a *poor preservative* in a weakly acid medium and it is further stated that in the pr in which benzoate of soda is efficient as a preservativ food materials it may also be toxic in its effect on man

No less a bacteriologist than Professor Theobald S shown that various types of bacteria (B. coli, paracolon proteus) are inhibited by benzoate of soda in neutral proportions varying from 0.3 to 0.5 of one per cent.[1] B soda thus exerts a marked preservative action in conc much less than one per cent. Bacteria are, in general, n sensitive to acids, and benzoic acid, like all other fairly str is more effective than its neutral salts. In fruit food acidity, such as apple or apple-butter, the fruit acids t exert a strong anti-fermentative action for most kinds of For this reason only very low percentages of benzoate o necessary for preservation. When these low proportion ployed (0.15 per cent. or less of sodium benzoate) the taste acid is slight and for many persons unobjectionable. experiments no deleterious effects on the kidneys or on could be observed. Our subjects took as much apple- apple-sauce as they had appetite for (150-200 grams butter daily corresponding to four times the volume sauce.) The concentrations of sodium benzoate varied per cent. to 1.2 per cent. in the apple-butter. The u taste of benzoic acid in high concentrations is ample p against its excessive use and it is only from large qua high concentrations that any detrimental action is liabl

I have elsewhere published observations which show th benzoate is far from being a powerful preservative age capable, however, of serving a useful purpose in retarding tion, *even when used in concentrations much below those r quite inhibit bacterial growth in a fluid medium.* This i

[1] Professor Smith's observations, carried out for the U.S. D of Agriculture, have not yet been published.

which should not be overlooked in discussing the efficiency of benzoates as preservatives. When used in conjunction with steam sterilization (as is generally the case) this retarding effect on bacteria (including reduced gas formation) may suffice to confer preservative value. *There is no evidence whatever that in these low concentrations (0.1 per cent-0.15 per cent.) benzoate of soda acts injuriously on the normal human organism, whether used in acid or neutral media, provided the total quantity ingested be kept within reasonable limits.*

Two other features regarding the general conclusions of the Lucas paper may perhaps be worth touching on. The absurd statement is made that after taking benzoated cider *excessive amounts of hippuric acid were eliminated.* It is not clear what is meant by excessive amounts of hippuric acid, but the implication that the amounts observed were excessive must suggest an injurious action, of which there is, of course, no evidence whatever. The statement that when large quantities of proteid, fatty or alkaline material are ingested with sodium benzoate there is a diminution of the toxic action of the drug, is of course, a truism. It is unfair to base practical conclusions on the study of the action of benzoic acid on an empty stomach, since when taken as a food preservative the benzoate must always be present together with food. The presence of carbohydrates, or fruit substances, helps to protect the stomach against the injurious action of any substance which in excessive quantities acts as an irritant.

I desire also to call attention to the following facts:

a. The toxic action of sodium chloride to living animal protoplasm is greater than that of sodium benzoate. (I have shown this for tadpoles; Gerlach has shown it for the mammalian heart.)

b. The human organism is provided with a fundamental mechanism for disposing of benzoic acid in a physiological manner. The powers of the mechanism are far in excess of the ordinary requirements of the human organism, including even the disposal of benzoates in preservatives.

c. The amount of energy required to couple benzoic acid with glycocoll to hippuric acid is extremely small, the indications being that this synthesis is an enzyme reaction. Likewise the amount of glycocoll required to pair a few grams of benzoic acid daily to hippuric acid is insignificant in relation to the total nitrogenous metabolism of the body.

d. There is as yet no evidence that the organism is better off for maintaining the lowest possible hippuric acid output, than for maintaining a somewhat higher level of physiological activity.

e. Decayed foods are not *improved* by the addition of benzoates, except in the sense that fermentation may be checked in part. The effect is like that of steam sterilization, only less efficient. The color of vegetable foods

is not affected by adding benzoates, for in a neutral medium the acid effect is lacking, and in an acid medium the fruit acids suffice to maintain color. Decay in foods may be checked by benzoates, but their inferiority in consistence, histological structure, etc., cannot be masked.

The conclusions of the Referee Board with regard to the action of sodium benzoate are in accord with the best investigations on this subject. Anyone who wishes to know what very large doses of soduim benzoate and benzoic can be taken without injurious effect should consult the paper of Lewinski[1] and the still more recent careful studies of Gerlach[2]. There is no soluble substance that can be taken into the digestive tract which does not possess toxic properties in some degree when given in sufficiently large quantities or concentrations. Benzoates and benzoic acid are, of course, no exceptions to this general rule.

The government can safely be guided in its regulations by the conclusions of the Referee Board and this holds true despite the fact that someone may occasionally bring on himself injurious consequences from the gormandizing and greatly excessive use of articles of food preserved by the use of benzoate of soda or benzoic acid. Certain dyspeptic people who ought to avoid fruits and acids in general ought probably also to avoid food containing free benzoic acid, just as they should avoid vinegar or spice, or even the free use of salt. This consideration, however, does not alter the fact that benzoate of soda used as a food preservative is singularly lacking in harmful effects on the human organism and that ill effects from its reasonable use have not yet been demonstrated.

[1] *Loc. cit.*
[2] *Loc. cit.*

Experimental Variation of Intestinal Flora by Changes in Diet.

By

Christian A. Herter (New York),

Professor of Pharmacology, Columbia University, New York

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During the past year I have been engaged in experiments designed to determine the influence of certain kinds of food upon the bacterial flora of the intestine. These experiments have been carried out mainly upon Rhesus monkeys and to some extent on human beings and kittens. Some of the results obtained have already been reported very briefly by my associate, Dr. A. I. Kendall, and are about to be published in extenso in a paper by myself and Dr. Kendall. I propose here to give a statement of the latest results deducible from our studies, without entering into full details. These results appear to me to be significant for the physiology of the digestive tract and it appears likely that they find application in certain therapeutic endeavors to modify the bacterial flora under pathological conditions in man.

In our studies we employed well-defined if not extreme types of food and made use of abrupt alternations in these different types. For example in our experiments on kittens an exclusive meat diet was employed for a period of one or two weeks and was then suddenly replaced by a diet of milk to which ten grams of dextrose was added daily, thus substituting a dominant protein diet for one in which carbohydrates preponderated. Similar experiments were made on man, though in somewhat less extreme form. In the experiments on monkeys the protein diet was represented by hard boiled eggs from which, after a period of one to two weeks, an abrupt change was made to a diet con-

si[illegible] of milk to which dextrose was added to the extent of ten grams or somewhat more daily. After a period of one to two weeks on the dextrose-milk diet there was an abrupt return to the diet of hard boiled eggs.

These experimental alternations in diet were found to be followed by definite and consistent changes in physiological conditions in three distinct directions: (A) in the nature of the intestinal bacterial flora; (B) in the putrefactive products of the feces and the urine; (C) in the clinical conditions.

A. The nature of [illegible]inal bacterial flora.

The chief charact[illegible]acterial flora on a diet which is dominantly protein in[illegible]e development of a strongly proteolyzing type of bac[illegible]isms. This is shown by the fact that the mixed fecal [illegible]a[illegible] such a diet, whether in the case of man or monkey or cat, [illegible]ontains a high proportion of bacteria capable of proteolyzing [illegible]in actively and of liquefying gelatin. The organisms have n[illegible] studied in the fullest detail individually, but by means of t[illegible]ual plating procedures it has been possible to show that on [illegible]rotein diet there is a large proportion of organisms which may [illegible]e classed in the B. subtilis group and which we may designate as subtiloid in character. It is doubtless to these that the active proteolyzing action of the mixed fecal bacteria is largely due. Careful studies were made in the case of the monkeys upon the influence of the protein diet on organisms of the B. aerogenes capsulatus (B. perfringens) type. It was found impossible (in the cases examined) to demonstrate the presence of organisms of this type in monkeys fed, even for a long period, exclusively on eggs. In kittens, on the other hand, and in man organisms of this type were regularly detectable and may have had a part in the proteolyzing action to which reference has been made.

A second noteworthy fact relating to the action of the mixed fecal bacteria on the protein diet is that the organisms possess the power of forming an abundance of gas in dextrose bouillon, lactose bouillon and saccharose bouillon. On twenty-four hours' sojourn in the incubator at body temperature the percentage of gas in the closed arm of the fermentation tube may amount to sixty or eighty or even ninety per cent. This is a very characteristic property of the flora upon a protein diet. Its explanation

is as yet not entirely clear. It does not depend on the types of bacteria which have been mentioned, since in the case of the monkey the subtiloid organisms (producing little or no gas) are not associated with B. aerogenes capsulatus. This high gas formation seems due to a peculiar symbiotic condition of the bacteria which we have been unable as yet to reproduce satisfactorily in an experimental way.

On changing from the protein to the carbohydrate diet the bacteria undergo pronunced changes which result in a gradual but rapid and definite substitution of an acidophilic, feebly proteolyzing type of flora for a strongly proteolyzing type. Just after the animal is changed from protein to carbohydrate there develops a transitional flora characterized by two noteworthy features, first a decline in the size of the bacteria and an impaired ability to take the usual stains. Evidences of degeneration are seen in the bacteria, particularly vacuolization. In some instances spore formation has been observed. Marked irregularities in distribution of the various organisms occur and are apparently due to the antagonism which has arisen between the outgoing protein flora and the incoming carbohydrate flora. As the latter gradually become dominant, the Gram stained fields, which, on the protein diet, were heterogeneous in appearance, tend to become more homogeneous and the most prominent organisms are Gram-positive rods thinner than those noted on the protein diet and somewhat longer. These rods are so abundant that the fields resemble strikingly those of normal nurslings. Cultural investigations have shown that these organisms are in reality allied to those characteristic of normal nurslings. A large proportion of the organisms present in the established dextrose-milk feces have the power of growing in broth of a high grade of acidity; they are, in fact, acidophilic bacteria. These bacteria grow relatively poorly on neutral plain broth. The mixed flora on this diet of carbohydrate and milk have little or no proteolyzing action though they are capable of growing slowly in milk and of inducing coagulation slowly. These organisms also grow very scantily in gelatin, which the acidophilic bacteria do not liquefy. The few liquefying colonies sometimes seen consist of the proteolytic bacteria remaining over from the protein period.

The mixed fecal flora from the carbohydrate dextrose milk diet produce very little gas in the sugar bouillon fermentation

tubes. Such fermentation tubes may, indeed show a complete absence of gas production. The reason for this appears to be the dominance of the acidophilic, non-gas-producing forms which crowd out the few remaining representatives of the gas-forming bacteria present in the protein period.

It should be said that the results obtained in the case of man have been less pronounced as regards the extreme alternations from proteolytic to acidophilic flora, although the changes observed were of the same general character. The variations in gas production were also much less extreme than those which were observed in the animal experime[illegible] The reason for this is perhaps to be found in the fact that it was not practicable in the case of man to carry on such extreme types of feeding as in the case of the animals.

B. Putrefactive products of the feces and the urine.

A well marked feature of the protein diet is the occurrence of neutral or alkaline stools containing products of putrefaction of known composition. These products include indol, skatol, phenol and aromatic oxyacids. They also include indolacetic acid which I have found in the case of man to be very definitely increased both in the intestinal contents and in the urine through the use of a dominantly beef diet. In monkeys on an egg diet no indolacetic acid was detected, either in the intestinal contents or in the urine.

Another product of putrefaction regularly present in the feces on a protein diet is hydrogen sulphide; that is to say it is possible to liberate hydrogen sulphide from the feces through the addition of hydrochloric acid. The amount of hydrogen sulphide bound in the feces is very much smaller on the dextrose-milk diet than on the meat diet or the egg diet, although there may not be much difference in the actual amount of protein intake in the two cases. The indol, skatol and phenol are regularly greatly decreased in the feces by the transition from the protein diet to the dextrose milk diet. It was frequently impossible, in fact, to obtain even traces of these substances during the dextrose milk period. Wholly in accord with these results is the fact that the indican and the aromatic oxyacids of the urine are markedly diminished during the dextrose milk diet and may, in fact, become undetectable. The dilution of the urine on a milk diet is in part responsible

for the lesser concentration of the putrefactive substances but only in part. It is easy to show that there is an absolute marked decrease in the amounts of these substances present on the dextrose-milk diet.

C. Clinical conditions.

The clinical conditions have been perhaps most significant in the case of the experiments upon monkeys where we were able to feed exclusively on hard boiled eggs for considerable periods of time. In these animals, as the proteolytic bacteria become dominant in the alimentary canal, a state of drowsiness develops. The animal rests on its perch, holding its head in its hands. It grows stupid and responds poorly to outside stimuli. It shows less interest in its food and in general shows but little interest in its surroundings. The animal, even after a hearty meal, sometimes spends considerable time in biting the woodwork of its cage. As the diet is changed to dextrose-milk both the psychical and physical attitude of the animal undergo a marked change. The monkey no longer holds its head in its hands; the posture becomes erect and the usual brightness and alertness return. The eyes lose their dull appearance and become bright. The animal also ceases to chew the woodwork of its cage. The development of the first group of symptoms just described when the animal is put back on the egg diet is so striking a feature that it cannot fail to impress the observer. It seems safe to conclude that after a variable period on the egg diet (ordinarily about a week) the animal becomes distinctly uncomfortable and loses its sense of well-being. The manner in which it sits holding its head in its hands with the head bowed down is an apparent indication of both mental and physical distress. In some monkeys these symptoms of depression are relatively slight, but they are seldom absent if an abundant diet of egg be consumed over a period of one or two weeks.

I am strongly disposed to regard the symptoms observed on the protein diet as signs of intoxication brought about through the excessive and unbalanced use of protein. It seems probable that this intoxication is facilitated by the presence of the actively proteolyzing bacteria which I have described as characteristic of the protein period of diet. Even in cats the signs of clinical differences on the two diets are not wanting, although here there

I am unable as yet to state to what extent the facts noted can be applied to pathological conditions in man. It is not likely that they are directly applicable, and it is doubtless true that varied researches would have to be undertaken to decide in what way the typical adaptations which I have here sketched are modified by the existence of inflammatory conditions, bacterial infections or other pathological states.

Résumé.

Ein Wechsel in der Diät, wie z. B. der Uebergang von einer vornehmlich aus Eiweiss bestehenden Kost zu einer solchen, vornehmlich aus Kohlehydraten bestehenden, hat allemal drei verschiedene Folgen, die sich beziehen auf: a) Veränderungen in der intestinalen Bakterienflora, b) Veränderungen in den Fäulnisprodukten in Urin und Fäzes, c) Veränderungen in den klinischen Erscheinungen.

Observations on Intestinal Infantilism.

By

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I desire here to call attention to a form of intestinal disorder associated with arrest of development which has as yet hardly received the attention which it deserves. For more then twelve years I have been aware of the existence of the type of disease to which I refer, but it is only recently that I have had an opportunity to work carefully at the pathological conditions involved. So far as I am aware, the condition of intestinal infantilism had not been recognized as a pathological entity by English or American investigators previous to my publication 1908[1]). In Germany, however, a condition of disease in children has apparently been clearly recognized for many years by Professor Heubner[2]) of Berlin, although he did not publish on the subject until about the time of my own publication. It seems probable, also, that some of the cases imperfectly described by Schütz[3]) are of the same nature as those described by me under the name of intestinal infantilism and by Heubner under the name of severe intestinal insufficiency in children above the age of nurslings.

It is not my intention to enter into anything like a full description of intestinal infantilism in this place, but rather to briefly state the leading characteristics of the disease, which stamp it as a definite pathological entity, and furthermore to discuss

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1) On Infantilism from Chronic Intestinal Infection. The Macmillan Co. 1908. Translated also into German by Dr. Ludwig Schweiger. Franz Deuticke. Leipzig u. Wien. 1909.

2) Ueber schwere Verdauungsinsuffizienz beim Kinde jenseits des Säuglingsalters. Jahrb. f. Kinderheilk. LXX. S. 667. 1909.

3) Chronische Magendarm-Dyspepsie im Kindesalter. Jahrb. f. Kinderheilkunde. LX. S. 794. 1905.

some special features about which there exists a difference of opinion between Professor Heubner and myself.

The condition of intestinal infantilism has its beginning usually in the second, third or fourth year of life and may have its onset in acute or subacute catarrh of the intestine and an enterocolitis; or it may perhaps develop insidiously and without definite warning. It is doubtless correct to regard the disease in its fully developed form as a rare one, although it must be true that in a large country, like the United States, a considerable number — probably many hundreds — of instances exist. Without making any special effort to bring to light a large number of these cases, I have now observed ten of them, and I know of a number of other instances in the practice of my colleagues which are almost certainly of the same nature as those which I have studied. The circumstance that the disease is nearly always seen among the children of well-to-do persons is probably to be explained, as suggested by Professor Heubner, on the supposition that the less well protected children of the poorer classes do not long survive, if they develop this disease.

In every case that has come under my observation there has been a history of periods of disturbed nutrition with loss of weight alternating with periods of improvement in nutrition and gain in weight. These variations ended in each case in a state in which there was apparently a complete arrest of the developmental processes. Although the most obtrusive signs of digestive disturbance such as diarrhoea and flatulence were in many instances measurably controlled by suitable treatment, there yet remained an arrest of growth and the utmost difficulty was experienced in bringing about any noteworthy increase in weight and improvement in nutrition.

I have divided the clinical characteristics of intestinal infantilism into two groups — a major and a minor. In the group of major, or essential clinical features are 1. arrest in the development of the body; 2. various obtrusive disturbances referable to the intestinal tract, especially large, characteristic fatty stools; 3. marked abdominal distension, due to dilatation of the colon and the accumulation of gas; 4. the rapid onset of physical and mental fatigue, the former being associated with flaccidity of the muscles; 5. the development of a moderate grade of anaemia; 6. the maintenance of a good grade of mental power and fair physical development of the brain, as indicated by the size and shape of the skull. As

examples of the physical arrest of development I may instance one of my patients who at seven years weighed only twenty-five pounds and had a height of only thirty-six inches, and also another case in which the patient — a boy — though sixteen years of age weighed only forty-four pounds and had a height of only forty-three inches. The character of the intestinal disturbance varies somewhat in different instances but the occurrence of abundant soft stools containing an excess of neutral fat and an abundance of soaps of calcium and magnesium is characteristic. The movements are not, however, always of this character in the same case but show considerable variation, with, however, a strong tendency for the characteristics just described to recur at short intervals. The excess of fat is often sufficiently great to constitute a condition of true steatorrhoea, if thereby we imply a loss of fat serious to nutrition. Pronounced watery diarrhoeal attacks occur in some instances but I think they may be said to be comparatively rare. In one of my cases there was no history whatever of diarrhoea in the ordinary sense, although the voluminous gas- and fat-holding stools were a feature which existed in alternation with constipation and apparently nearly normal movements. During the time when the characteristic voluminous stools are obtrusive the patients are in especially poor condition and show a loss of weight together with disturbances in peripheral circulation, which are shown by cold hands and feet and a pinched and sometimes cyanotic appearence in the face. These disorders, though often of short duration, may cause a good deal of prostration and in severe cases the patients are so much enfeebled as to get about only with greatly increased difficulty. When the diarrhoea ceases there is a tendency to rapid recovery, although a loss in weight of half a pound or a pound suffered during a few days of pronounced digestive disturbance may not be regained for several weeks.

Among the minor or accessory clinical features which have been observed by me in intestinal infantilism are to be included slight indications of rickets, excessive sweating about the head during sleep, irregularities of appetite which is sometimes excessive, increased thirst, increased volume of urine and various signs of nervous instability. A subnormal temperature with cold and pale hands and feet may commonly be observed. The skin is apt to be dry and rough, but this latter manifestation is one that is corrigible by means of suitable bathing and rubbing. The tongue

is apt to be somewhat more red than normal and the papillae swollen but there is no tendency to the development of "geographical tongue". The entire tongue may be slightly swollen and may present indentations from the teeth.

Considerable time has been devoted to the study of the bacterial flora of the intestine in cases of infantilism of the type under consideration. As a result of these studies it becomes evident that it is necessary to distinguish between the conditions present during what one may call the active stage of the disease and those present in the later stages. Under the latter conditions where the abnormal processes have lasted for a long period of time — say eight or ten years — the bacterial conditions may show nothing characteristic or definitely pathological despite the fact that there exists a great arrest of development. On the other hand if we study our patients during the active period in which the characteristic disturbances of digestion occur, we can count on meeting types of bacterial flora which so far as I am aware we do not meet in the same numbers and combinations either in normal conditions or in other disorders of digestion. The methods of study which have proved most serviceable in this investigation have been the study of the Gram-stained fecal fields; second, the study of the sediments of the saccharose, lactose and dextrose bouillon and plain bouillon fermentation tubes after inoculation with the mixed fecal flora; and third, a variety of aerobic and anaerobic cultural procedures involving the use of many kinds of media made necessary by the difficulty of growing certain species of bacteria on ordinary media. A striking feature in well-marked cases is the preponderance of Gram-positive microorganisms. Study showed that the organisms in question corresponded to the B. bifidus of Tissier, the B. acidophilus of Moro and a hitherto apparently undescribed organism which we have called B. infantilis. It is not my intention here to enter into a detailed description of the flora found in cases of intestinal infantilism. It will suffice to indicate certain special points which it seems desirable to emphasize.

The presence of B. bifidus in the intestinal contents of our cases of infantilism is indicated in part by a study of the sediments of the sugar bouillon fermentation tubes. The inoculation of the mixed flora into the sugar bouillon fermentation tubes leads commonly to an abundant or more scanty growth of bifid organisms which can be shown to have all the morphological and cultural

characteristics of the organism first described by Tissier. It is not possible to state to what extent the intestinal contents may consist of B. bifidus which exists there only seldom in the bifid form and generally in the form of plain rods with narrow or pointed ends. The organism which we have named B. infantilis is probably present very abundantly in most or all cases of well developed intestinal infantilism. It occurs as a Gram-positive rod, agreeing closely in size with the plain form of Tissier's B. bifidus. It is not clear that it differs materially from the latter in any morphological characters. The organism has the peculiarity of growing aerobically on suitable media and forming spores. Many cultures have the further peculiarity of forming a pellicle from which may be extracted a mucinous and possibly also a fatty material.

I cannot pass over without comment the strong possibility of a relationship between our B. infantilis and an organism very recently studied and described by H. Noguchi[1]) as a phase of B. bifidus. Noguchi, starting with branching organisms apparently of the bifidus type, suceeded in cultivating an aerobic, spore-forming, pellicle-producing culture which corresponded very closely in details to our B. infantilis. By a gradual training of these organisms to anaerobic life, Noguchi was able to bring about a complete reversion of the aerobic phase of this bacillus into an anaerobic phase — results which one would be inclined to regard as almost unbelievable excepting when occurring in the experience of so highly trained and experienced a technician as Dr. Noguchi. These results he obtained by cultivating the bacillus semi-aerobically and then abruptly diminishing the quantity of oxygen after three or four successive cultures. The details of this reversion as described by Dr. Noguchi will well repay study. Not the least interesting conclusion drawn by Dr. Noguchi is that B. bifidus of Tissier corresponds in its aerobic phase with B. mesentericus (fuscus). Noguchi calls attention to the fact that B. mesentericus is one of the most widespread saprophytes and is constantly found on the surface of the skin. He considers the only source of B. mesentericus, that is so say, bifidus, in the stools of breast-fed infants to be the breast itself.

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1) Pleomorphism and Pleobiosis of Bacillus Bifidus Communis. Journ. Exper. Med. XII. p. 182. 1910.

I am not disposed to doubt the validity of these conclusions of Dr. Noguchi which may prove of considerable practical importance in the interpretation not only of the flora of intestinal infantilism but of the intestinal tracts of young children in general. Dr. Kendall was inclined to class B. infantilis with the Subtilis group rather than with B. mesentericus but it is perhaps true that the very slight degree of liquefying power observed in this organism would make it more appropriate to class our organism in the Mesentericus group than in the Subtilis group. The chief difficulty which I see in an agreement of our results with those of Noguchi is that B. infantilis exhibits so few characteristics of ability to induce even slight putrefactive decomposition with formation of indol, hydrogen sulphide, etc. If it should prove to be the case, as seems to be probable, that B. infantilis represents simply one phase of B. bifidus and that both these organisms are essentially members of the Mesentericus group, it will not merely clear up some puzzling features in the bacteriology of the digestive tract but will emphasize still more strongly than I have been able to do the great numerical importance of B. bifidus in cases of intestinal infantilism. I have suggested that in our cases of infantilism perhaps the most striking feature is the persistence of bacteria characterizing the infantile period of life, into a period extending many years beyond this. The work of Noguchi seems to me to help to substantiate the correctness of this view.

Besides the bacterial features mentioned there are two others which deserve a passing word. First, coccal forms are sometimes very abundant. Their numbers, however, vary a good deal in the same case at different times. They are commonly Gram-positive and the diplococci and coccal bacilli may correspond closely in morphology and cultural characteristics to the enterococcus of Thiercelin or the Micrococcus ovalis described by Hirsch-Libmann. The relation of these organisms to the others in the digestive tract is not yet clear. The second point which I would mention is that in well-marked cases of infantilism the organisms of the B. coli group may be wholly deficient in the stools or present only in very small numbers. This is a peculiarity which is certainly noteworthy in children beyond the infantile period of life. A phase of this peculiarity to which I attach considerable importance is the reappearance of organisms of the B. coli type coincident with the gradual recession of organisms of the Bifidus and B. infantilis types.

In my original publication on infantilism I pointed out that the two leading features, distinct but related, of intestinal infantilism which must be considered in any endeavor to picture the morbid processes on which the clinical manifestations depend are, first, extreme retardation in the general bodily development, and secondly, the state of intoxication which manifests itself in well marked derangements of the neuro-muscular system. As regards the retardation of development I think I have been able to bring forward sufficient evidence to support the contention that the arrested growth can be explained by the inability of the organism to secure an adeqnate supply of nutrient material from the contents of the digestive tract. Careful balances indicate that the failure to absorb sufficient calcium and magnesium accounts for the arrest of skeletal growth, while the restricted absorption of carbohydrates and fats explains the failure to store fat, and at least partially accounts for the cessation in the growth of the muscles. The proportionate slowing in the growth of the viscera may safely be assumed to have a similar origin, although possibly here the physiological adaptation of visceral structures to the needs of the rest of the body may have an influence. The relatively large development of the head and brain may be dependent in part on the large size of the brain at birth, but it seems that the growth of the brain in infantilism is distinctly out of proportion to the very slow growth of other parts of the body.

It is seen that the cardinal features of infantilism, the prolonged arrest of development in infancy and early childhood, may be attributed mainly to an impaired power of absorption of fats, salts and proteins. Between the absorption of fats and the absorption of the salts of calcium and magnesium there appears to be a close relationship. It has been mentioned that the characteristic stools in infantilism contain considerable quantities of the soaps of calcium and magnesium and it may well be true that the failure to absorb these soaps is one of the most inportant elements in that deprivation of mineral constituents which is the basis of the retardation of the skeleton. The relation between insufficient carbohydrates and defective absorption appears to be very remarkable, for the restriction in this class of food stuffs is one often encouraged by the physician on account of the evil consequences often following even the moderate use of sugars and starches. Very likely the inability of the intestine to completely absorb the dextrose

formed during the digestion of starches is a factor in robbing the organism of a proper share of carbohydrates, but to this factor is certainly added the loss occasioned by the excessively rapid decompositition of dextrose by acid and gas-producing bacteria. I have attributed the impaired power of absorbing food stuffs from the intestinal tract to a chronic inflammatory process involving especially the lower portion of the colon. I have based this view on the occurrence of mucus, epithelial cells and leucocytes intimately mingled with the intestinal contents. The existence of an inflammatory condition in the intestine is questioned by Heubner who is disposed to regard the state of the intestine as one of functional insufficiency and atony without definite inflammatory origin. I admit that there are periods in the history of cases of infantilism in which the indications of inflammation are slight and unobtrusive, but I believe that these periods represent times of temporary subsidence of a persistent, chronic inflammation of varying intensity and distribution. Heubner questions the ability of normal intestinal bacteria to bring about intestinal inflammation in older children and does not consider it clear that the hypothetical inflammation of the intestine could lead to so severe a disturbance as this of infantilism.

I do not pretend to be able to satisfactorily define the role of the disturbed bacterial conditions in the production of infantilism. It is conceivable that the bacterial conditions may be regarded as secondary, wholly accidental and therefore in no sense causative. There is, however, one very important consideration which should not be overlooked in this connection. This is the fact observed in many of my cases and admitted by Professor Heubner for several of his cases, that the onset of the state of infantilism is often consecutive to acute or subacute disorders of digestion in which it would appear that bacteria play a very definite part and are the occasion of definite inflammatory conditions. I make no claim that the organisms of the Bifidus type, including, perhaps, B. infantilis, are definitely pathogenic in the accepted sense of that word, but am strongly inclined to maintain that these organisms have, perhaps in part through their acid-producing properties acted as mild irritants capable by their persistence and wide extent of initiating disturbances in function. I have also to own that in ascribing to bacterial and to chronic variable and mild inflammatory conditions an important role in the pathology of infantilism, I am influenced by my experience with chronic inflammatory conditions in adults.

I believe there exists definite evidence at present only imperfectly studied, to the effect that chronic ileocolitis associated with a preponderance of abnormal types of bacteria is responsible for many chronic disorders of nutrition characterized by gradual shrinkage of the general bodily tissues including the muscles and skeleton.

I do not, however, care to pursue this controversial point further at the present time, and I shall conclude what I have to say about the pathology of intestinal infantilism by reference to the part played by the products of abnormal intestinal decomposition in bringing about certain toxic symptoms which occur in the course of the disease. On this point also Professor Heubner has expressed scepticism. My experience leads me to think that the element of intoxication is very variable in different cases of infantilism, being in some examples of the disease characteristic, extending over years in unmistakable intensity and definiteness, and in other cases being a subordinated or relatively unobtrusive condition at least during a portion of the time. The patient in whom the toxic symptoms are well defined may show these manifestations in a variety of ways. The most prominent of such symptoms are irritability of the nervous system shown in emotional over-action and heightening of the reflexes. This emotional over-action is apt to alternate with mental and emotional depression. Circulatory disturbances are common, the face being sometimes flushed and the lids swollen and drooping; more frequently, however, the face is pinched. There is at times a little rise in temperature which apparently can be explained only on the supposition of some kind of intoxication. Most striking of all is the rapid muscle fatigue which sets in after slight exercise. Indeed in many instances the muscles themselves becom thin and flabby.

It appears to me that these conditions can only be regarded as having a toxic origin. Their great variability within relatively short periods of time is an additional confirmation of this view. Moreover it is precisely in the cases which show these peculiar disturbances of neuro-muscular function that we find most strongly marked the evidences of excessive and abnormal intestinal putrefaction. These evidences consist in an increase in the ethereal sulphates, indolacetic acid, aromatic oxy-acids and volatile phenolic substances. It is true that not all these need be present in the same case. I consider the rise in the ethereal sulphates and the excess of aromatic oxy-acids the most constant features of excessive putre-

faction in the active stage of intestinal infantilism. The presence of considerable indoxyl potassium sulphate is a variable condition — very marked in some cases, much less marked in others and often highly variable in the same case. In some instances great excess of indolacetic acid is a continous feature. It shows, as I have already pointed out, as the urorosein of the urine.[1]) I am inclined to associate marked muscular prostration with a constantly striking excess of indolacetic acid or with extreme indicanuria in the urine. Particularly noticeable in this connection is the fact that these various nervous symptoms tend markedly to retrogress when there is a recession in the formation and excretion of the substances I have mentioned. Another point which I would emphasize strongly because I think it is too much overlooked by clinicians and pathologists, is the fact that it has been shown that indol in very small quantities is capable of inducing rapid muscle fatigue. I have not had an opportunity to study the influence of indolacetic acid in this relation but it appears in a high degree probable that it possesses a similar depressant action on the voluntary muscles. The similarity in chemical constitution to indol gives color to this view. It should be remembered also that while indol undergoes oxidation and is thus got rid of in the form of the indoxyl compound, indolacetic acid undergoes little or no oxidation in the body but is excreted as such, thus giving it opportunity to act upon the neuro-muscular structures.

For these reasons, which might be considerably elaborated, I maintain that the toxic symptoms observed in many of our cases of intestinal infantilism, have their most reasonable explanation in the action exerted by products of decomposition in the intestinal tract. I am not prepared to maintain that fever is caused by any of the substances mentioned, nor can I believe that it is due to inorganic salts, as some investigators claim. It seems more likely to be dependent on substances the nature of which have as yet escaped attention.

As regards the therapeutic modification of the conditions existing in intestinal infantilism, it is almost impossible to make a general statement applicable to all cases. The symptoms of the

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1) The Relation of Nitrifying Bacteria to the Urorosein of Nencki and Sieber. Journ. Biol. Chem. IV. p. 239. 1908. Also: On Indolacetic Acid as the Chromogen of the „Urorosein“ of the Urine. Ibid. IV. p. 253. 1908.

disease vary so much in different cases and from day to day in the same case, that it is necessary for the physician to gain his own experience in the application of the therapeutic principles that emerge from a rather complex combination of processes. The environmental requirements in these cases are very well defined. They call for a temperate, equable and sunny climate in which the patient can live out of doors, and secondly they call for quiet and soothing human surroundings and limited companionship. Dietetic measures are of course the keystone of the therapeutic situation in this disease. Without the closest attention to them there is small chance of favorably modifying the intestinal processes which underlie the condition. Cautious and prolonged observation and experiment are necessary to secure the best dietetic conditions in each case. The task of the physician is to secure the absorption of food stuffs adequate in quality and quantity for a moderate growth of skeleton, muscles, etc. with as little waste as possible from non-absorption and as little opportunity as possible for excessive putrefaction. The intelligent restriction of the carbohydrates is an absolutely essential feature in the management of these cases. Rice appears to be one of the best forms in which carbohydrates may be allowed. Of equal importance is the careful regulation of the fats. The guiding principle here consists of giving only so much fat as will be in large measure absorbed. It is desirable to avoid the prolonged formation of excessively fatty stools. The main objections to such an excessive fat loss are two: the unnecessary and uneconomical waste of calcium and magnesium soaps and the interference with digestion and absorption occasioned by the presence of fatty material in excess. The proteins are better tolerated than either fats or carbohydrates but it is necessary to experiment in order to see what form of proteid is best utilized. Some children do badly on eggs, although most of them tolerate eggwhite. Beef, mutton and chicken, or broths made from these, are usually well taken. The necessity for cutting down the carbohydrates and fats in the treatment of infantilism leads to a corresponding tendency to increase the protein above the ordinary proportions in food. This is objectionable in favoring excessive intestinal putrefaction. This undesirable tendency may be in a measure overcome by the free use of gelatin which is capable of replacing the carbohydrates and fats within moderate limits. The chemical constitution of gelatin is such that it does not give rise to indol, skatol, indolacetic acid or

aromatic oxyacids. This fact can be utilized in this connection. I believe I have made use of this form of food in a highly advantageous manner in several of the cases of intestinal infantilism which I have closely studied.

It is difficult to give definite rules in regard to the use of milk. The experience of Heubner is apparently strongly against the use of milk and the fact that in this disease we seem to be dealing with the persistence of certain modified flora of infancy — flora growing readily in milk — may afford an explanation of the intolerance for milk which exists in many cases. My experience, however, has been that some children are able to tolerate considerable quantities of plain or fermented milk. Therefore I am not disposed to wholly abandon the use of milk without a careful trial. Butter-milk is in some instances very well tolerated and is apt to be a useful food, in the later stages of the disease where the inflammatory conditions have subsided and where we are confronted mainly with retardation in growth. I have used lacto-bacilline, milk and also tablets of lacto-bacillus, but have not been able to observe any special benefits from their use.

The pharmacological measures to be employed in the course of the treatment of infantilism are so subordinated to those of environment and diet that it seems hardly necessary to discuss them here. It seems in general best to avoid the use of carthartics. The employment of iron and arsenical tonics by mouth appears to be of doubtful utility, especially during the active period of the disease. The use of cacodylate of soda subcutaneously might be worth trying where there is considerable anaemia. The possibility of employing eserine to reduce congestion in the digestive tract is perhaps worth more consideration than it has received.

It should be entirely clear that the state of intestinal infantilism is a highly refractory one and not likely to be followed by a return to normal growth except as the result of discriminating therapeutic interference. Among the poorer classes there are probably a good many deaths from the acute or sub-acute infections which lead to infantilism, but among the well-to-do population who are willing to take pains with their children, deaths are very few either in the initial acute and sub-acute stages or in the later stages. It is to be expected that a permanently undersized individual will be the outcome even in the most favorably progressing instances of the severe form of infantilism. In one of the ten

instances that I have followed there was excellent compensation after the tenth year so that the outcome was a young adult of normal size. In this case the retardation of growth was almost complete during four or five years. In a good many cases the outcome is a state of marked dwarfism and there can be little doubt that a certain proportion of dwarfs in the community arise through the persistence of intestinal infantilism. Aside from cretinism it may be that this is the most common cause of dwarfism. It is important to note that the retardation in physical development is not incompatible with a high degree of mental culture.

It is also to be remembered that temporary relapses are very common in the course of infantilism, even when the greatest care is being taken to prevent them. Such relapses on the part of the digestive apparatus may occur while the patient is upon a diet which has been well tolerated for weeks or even for months. The most frequent cause of relapse is the attempt to encourage growth through the too free use of carbohydrates. When the relapse occurs the feces become voluminous, lose their conglomerate appearance, show an excess of fat or an excess of acid due to the fermentation of carbohydrates. In persistent relapses an increase in the number of organisms of the B. infantilis and B. bifidus types can often be determined.

It should be evident that treatment of these cases of infantilism requires the utmost tact and patience on the part of the physician and the intelligent confidence of the parents. For obvious reasons it is of the first importance that the physician should recognize the nature of these cases at the earliest possible time in order that he may prepare the parents for the long, perplexing and often discouraging course of events which necessarily attends even the most favorable progress of these unique types of digestive disorder.

Résumé.

Nach der Schilderung des klinischen Krankheitsbildes des intestinalen Infantilismus wird über die Ergebnisse der Untersuchung der Bakterienflora des Darmes bei dieser Krankheit berichtet. Im Stadium der akuten Darmstörungen findet man eine Bakterienflora, wie man sie kaum unter normalen oder anderen pathologischen Verhältnissen antrifft. Diese Flora wird genauer beschrieben. Die

Ursache für die Wachstumsstörung ist die Unfähigheit des Organismus, genügend Nährmaterial aus dem Darminhalt aufzunehmen. Das betrifft vor allem die Fette, Eiweissstoffe und Salze. Daher rührt auch die eigentümliche Beschaffenheit der Stühle. Kohlehydrate werden gleichfalls schlecht ausgenutzt, zum Teil durch Bakterien im Darm zerstört. Die Ursache der Darminsuffizienz muss vor allem in einer chronischen Schleimhautentzündung von wechselnder Intensität gesucht werden. Zu alledem gesellen sich toxische Wirkungen. Darauf deutet auch der Harnbefund hin. Zum Schluss wird die Therapie der Krankheit besprochen.

NAME	DATE DUE
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